Using Koi Carp to Produce Fish Silage

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by

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ABSTRACT

The natural environment of New Zealand is a highly cherished and important factor of the country. Introduced koi carp is considered a pest in New Zealand because it has a deleterious effect on fresh water systems. Hence eradicating the fish is essential. Identifying possible uses for this captured fish will help offset the cost of eradication.

The aim of the study was to identify feasible products that could use koi carp as a raw material without the added impediment that a long term stable supply would not exist once fish numbers had been drastically reduced. The applications identified from a literature search could be categorized into three main groups: as food (for example canned carp); processed non-food uses (for example extracting fish collagen); and miscellaneous applications (such as biofertilizer). Silage production is a simple and cost effective method for using whole koi carp. The process involves mixing thawed minced fish with an acid and keeping it at a particular temperature for a short time.

The effect of stirring conditions, pH and temperature was determined in the preliminary laboratory trials. A good silage requires a constant pH between 3.5 and 4.0 throughout the process and needed to be kept at 40°C for four days. Throughout the process the mixture needs to be thoroughly mixed. The main trials investigated the effect of different mineral and organic acids (singly and in combination) and the effect of using kiwifruit pulp as a source of proteolytic enzymes.

The combination of hydrochloric acid and acetic acid (50:50 v/v) gave the best silage with high soluble solids content. Adding kiwifruit gave higher soluble solids after 36 h but the proteolytic activity then stopped. The process may need daily additions of green kiwifruit pulp to obtain a good silage.

It is recommended that further studies on the biochemical changes during the silage process, the effect of other acid combinations in different proportions, the effect of acid strength, and other sources of exogenous proteolytic enzymes be investigated. It is also recommended that the costs of a commercial process be determined.

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1: OVERVIEW

1.1. Importance of the environment to New Zealand

New Zealand is known for its natural beauty, which includes mountains ranges, unpolluted sandy beaches, lush rainforests, rivers, lakes, glaciers, extinct and active volcanoes, and above all, its unique flora and fauna. After 1000 years of human settlement, New Zealand has undergone dramatic changes in its indigenous ecosystems and species (Northland Regional Council, 2002). Efforts are underway to restore what has been lost and to maintain what remains. One survey (Growth and Innovation Advisory Board, 2004), found that nearly 90 per cent of New Zealanders consider the environment very important to them, rating it the third most important aspect of New Zealand behind quality of life and quality of education. Customary values of the Maori people include the concept of guardianship of resources for the future generations.

New Zealand's economy largely depends on its natural environment. For example, about 65 per cent of New Zealand's electricity is from renewable sources. Products from farming make up a major part of the export earnings and advertising by New Zealand Tourism emphasises New Zealand's natural environment.

The fresh water systems in New Zealand are known for their cool clear waters and trout and salmon sports-fishery. They are valuable sources of drinking water, irrigation, and energy. Protecting the nation's fresh water resources is a growing challenge. These waters also contain unique native aquatic fauna and flora. Wetlands and rivers provide habitats for a variety of fauna, including birds, fish, frogs and invertebrates. Most of the fauna is endemic to New Zealand.

Out of the 50 fresh water fish species present in New Zealand, 22 species are introduced (Watts & Peters, 2010). Some introduced species are beneficial but some have had deleterious effects. Examples of introduced pest fish include koi carp, catfish, rudd, and Gambusia (also known as mosquito fish) (NZFWL, 1999). All these pest fish can degrade native fish habitats, as well as act as direct predators and competitors. It is essential to find control measures to prevent these

pests spreading and to eradicate them so the native aquatic fresh water environment is maintained.

1.2. Carp introduction to New Zealand waterways

Carp is one species introduced to the New Zealand waterways that can be considered a pest or (its legal designation) an 'Unwanted Organism'. Carp is an oily freshwater fish of Cyprinidae family and is native to Europe and Asia and includes common carp, silver carp and grass carp. The typical carp is in the *Cyprinus* genus and common carp is the most widespread and well-known member.

Koi carp are ornamental domesticated varieties of common carp and are believed to have originated in Japan (hence their other name Japanese carp). Koi carp is thought to have established in New Zealand in the 1960s when they were accidentally imported as part of a goldfish consignment. The main features that distinguish koi carp from gold fish is that they are larger and have two pairs of barbels at the corner of their mouth.

Koi carp is widely spread in New Zealand waterways but mainly found in the Auckland and Waikato regions and can make up 80 per cent of the total fish biomass in Waikato lakes and rivers (Watts & Peters, 2010). They are spreading into Northland and a containment area has been created between Auckland and Hamilton.

Carp are considered a pest because they directly and indirectly affect water quality and the aquatic ecosystem. They are opportunistic omnivores and feed on invertebrates, spawn and juvenile of other fish, and plants. They are benthivores, which means they feed at the bottom of lakes, ponds or rivers. They suck bottom sediment, blow out the food to wash it and suck in rinsed food leaving the unwanted material. As they feed, they uproot vegetation, increase water turbidity, and causes habitat loss for plants and native fish.

New Zealand waterways need to be preserved for future generations. Groups such as the Federation of New Zealand Aquatic Societies help prevent spread of these aquatic pests by increasing public awareness, especially hobbyists, of the

harmful effects of the pests. Eradicating koi carp can be helped if an economical use could be found for them.

1.3. Possible uses for carp

Koi carp could be a source of human food or used as animal feed. Products could be made from various fish components. For instance, collagen or gelatin could be extracted. The whole fish could be used for making bio-fertilizers or biogas. Another practical application is fish silage, which can be used in both the fertilizer and pet food industries. This process has various advantages such as requiring relatively low technology and facilities, unspecialized labour not, and a means of use the whole fish.

Fish silage is a liquefied product made from whole fish or fish parts. Liquefaction is caused by the enzymes already present in fish and may be enhanced by adding acid. The enzymes break the fish proteins into smaller soluble parts. The added acid prevents bacterial spoilage and speeds up enzyme activity.

Identifying the correct amount of acids (or combination of acid) makes the process economical. The pH and temperature affect enzyme activity and need to be optimised.

1.4. Thesis overview

Chapter two is a literature review of the major problems caused by carp, the possibilities for using carp in a useful way, and then goes on to give a detailed description of the silage process.

The experiments done to identify the effects of pH, temperature and acids on manufacturing fish silage are described in chapter 3 - Materials and Methods.

Chapter four presents and discusses the results obtained and identifies optimum pH, temperature and acid (or acid combination) for producing fish silage. Conclusions and recommendations are given in Chapter 5.

2: LITERATURE REVIEW

2.1. Maintaining New Zealand's Natural Environment

New Zealand is known for its natural beauty with mountain chains, volcanic regions, miles of coastline, rainforests, and lakes (Stecker, 2007). A major feature of New Zealand is its unique native biodiversity, which has developed because New Zealand separated from other landmasses about 80 million years ago (University of Waikato, 2007). New Zealand initially had only few native mammal species, dominated by bird species. Human settlement approximately 1000 years ago introduced many other animal species, which changed New Zealand's land, fresh water systems and marine environment.

The aquatic system, which includes marine and fresh water environments, are socially, spiritually, culturally and economically important to New Zealanders. The fresh water ecosystems provide habitat for over 38 native fresh water species, 38 native aquatic plant species, over 160 water bird species including five native ones and hundreds of aquatic and semi-aquatic invertebrate species (Chadderton *et al*., 2004). Many of these fish species are valued by Maori people as their traditional food source. However, many of the native plants, animals and their habitats are under threat from human activities and introduction of unwanted species. This loss and degradation diminishes the natural biodiversity as well as the quality of life of New Zealanders. Therefore, it is essential to conserve freshwater environments for the future generations, which is helped by Acts such as the Resource Management Act (1991).

New Zealand has over 50 fresh water fish species (NZFWL, 1999). About 35 species are endemic and over. 20 species are introduced fish (Watts & Peters, 2010). Nearly half the native fresh water species migrate to and from the sea during their life cycle. They are subjected to various threats during migration, as well as pollution caused by various human activities, habitat loss and threat from introduced species (NZFWL, 1999). Most of the fresh water exotic species were deliberately introduced to contribute to the pleasure and profit of the inhabitants and as part of a deliberate acclimatization policy (Champion *et al.*, 2002). Game fish such as trout are one example.

Introduction of fish species, aquatic plants and associated invertebrates continued unchecked until the later part of the. 20th century. The threat caused by introduced species is a major concern. These species compete with native species for food. For example, Galaxiids, a very primitive form of native fish now compete with introduced species for their main food- invertebrates. Other introduced species that have a devastating effect on native biodiversity and waterways include trout, koi carp, catfish, rudd, perch, tench, and Gambusia (mosquito fish) (NZFWL, 1999). Objections to introducing new fish species began to increase and legislation has been introduced to prohibit entry of undesirable species.

2.2. Carp and Their Environmental Impacts

Carp is an oily freshwater fish belonging to the family Cyprinidae, which is the largest fresh water fish family. Its members are known as cyprinids. Some cyprinid species such as common carp, crusian carp, grass carp, silver carp, and bighead carp are commonly called carp. *Cyprinus* is the genus of Cyprinidae and the most widespread and well known member is the common carp (McCrimmon, 1968). It originated in Western Asia and became naturally dispersed in China and Siberia. It also reached the Danube basin in Europe, and Romans farmed it and later transferred it to Western Europe. It spread further during 13th to 15th centuries (Billard, 1995).

Carp is one among the first fish species dispersed by humans. Common carp have been introduced to 59 countries, mainly for farming, sport fishing, commercial fishing and aquarium trade (Welcomme, 1992; Billard, 1995). Today, carp have become one of the 100 world's worst invasive alien species (ISSG, 2000) and is called an "exotic ecosystem engineer" (Crooks, 2002) as it has both negative and positive influences on availability of resources to other organisms via various mechanisms.

Common carp can tolerate a wide range of temperatures from 1 to 35°C. They also can tolerate rapid temperature fluctuations and a wide range of pH (Billard, 1995). During summer, due to the high photosynthetic activity, dissolved oxygen levels can fluctuate widely. Common carp can tolerate these fluctuations. They

can metabolise anaerobically and hence can survive supersaturation or anoxic conditions that occur in summer.

Carp are omnivorous and feed at all trophic levels. They feed on aquatic crustaceans, worms, insects, aquatic plants, algae and seeds (Osborne, 2006). Carp are also opportunistic feeders. They first feed on benthic organisms and when these are depleted start hunting for larger zooplanktons in open water (Billard, 1995).

Wild carp is slimmer than the domesticated form and can grow to a maximum 120 centimetres and reach a maximum weight of over 40 kg. Research conducted on common carp collected from brackish water pond in Pakistan revealed that carp constituted of 65.6% moisture, 9.7% of ash (dry weight), 22.7% lipid (dry weight) and 66.2% protein (dry weight) (Ali *et al.*, 2005).

Initially carp were an important food source for humans. Later it became very popular as an ornamental fish. The two most notable ornamental carp are goldfish and koi carp. Goldfish is the domesticated form of Prussian carp and koi carp is the domesticated form of common carp. Selective breeding of common carp in the 1820s in Japan resulted in the koi carp (Jordan, 2006). They are considered good luck and treated with affection in Japan. However, koi carp have become one of the least desirable species in the New Zealand fresh water fish fauna.

2.2.1. Koi carp in New Zealand

Koi carp has been introduced to New Zealand accidentally in 1960s as part of a goldfish consignment (Pullan, 1984) because of its superficial resemblance to goldfish. The characteristic feature differentiating koi carp (Fig. 2.1) from goldfish is the presence of barbels on koi carp carp lips, which are sensory organs that help koi carp search for food through touch and taste (Billard, 1995). Goldfish (Fig. 2.2) are smaller than koi carp and have a great variety of body shapes whereas koi carp have a common body shape and a wide range of coloration patterns. Koi carp weighing up to 12 kg (Osborne, 2006) and 700 mm long (Tempero *et al.*, 2006) have been caught in New Zealand.

Figure 2.1: Koi carp caught from Lake Waikare (with barbels)

Figure 2.2: Gold fish (No barbels)

Koi have similar durability to common carp and can tolerate temperatures from 0.7°C (Brown *et al.*, 2001) to 34°C (Hume & Pribble, 1980). However, prolonged low winter temperatures turn inhibit the koi carp's immune system. At low temperatures, the koi carp's digestive system nearly stops and they eat very little. They regain their appetite when the water warms up during spring.

Water temperature is an influential factor in spawning of koi carp. The ideal water temperature of common carp is 15°C - 17°C (Crivelli, 1981; Stuart & Jones,

2002; Tempero *et al.*, 2006). Fecundity of koi carp in New Zealand is similar to that in other countries (Tempero *et al.*, 2006), with the number of eggs produced being influenced by the size of koi carp. An average of 300,000 eggs can be produced annually and there are chances of multiple spawning at favourable water temperatures (Stuart & Jones, 2002).

Koi carp are omnivorous and eat anything from very tiny phytoplanktons and zooplanktons to small fish (Lammens & Hoogenboezm, 1991). The feeding habit of koi carp cause great damage to the waterways they are living in. This feeding habit is commonly described as being like a 'vacuum cleaner' and involves sucking everything from the bottom and spitting out what is not wanted through their gills (Zambrano & Hinojosa-Garro, 1999). Koi carp dislodge and uproot vegetation as they feed on benthic invertebrates, thus increasing water turbidity and destroying fish and plant habitats (Crivelli, 1983; Chumchal *et al.*, 2005; Driver *et al.*, 2005).

These harmful effects caused by koi carp pose a major threat to New Zealand natural aquatic systems. Wild stocks of koi carp were first found in Waikato River in 1983 (Pullan, 1984) and they are now widespread in the Auckland and Waikato regions. They are also spreading to many other regions of the North Island and have been found in isolated places in Wanganui, Hawkes Bay and Wellington (FNZAS, n.d.). They were first recorded in the Tasman-Nelson region in 2002 and an active campaign has been conducted against them by the Department of Conservation (NCC, 2009). To prevent koi carp spreading further, a containment area between Auckland and Hamilton has been created (FNZAS, n.d.). Recreational fishing is allowed within the containment area but all carp caught must be immediately killed.

The invasion of koi carp in New Zealand has been a major problem. As a result, the New Zealand government had a strategic policy goal since 1990 to eventually eradicate the species. The first step was to declare *Cyprinus carpio* and all hybrids a noxious fish species under Freshwater Fisheries Regulations (1980). The second step was to designate the species and its hybrids as "unwanted organisms" under the Biosecurity Act (1993). The Federation of New Zealand

Aquatic Societies also considers koi carp a pest and measures have been taken to prevent the species spreading (FNZAS, n.d.).

The structure and functioning of fresh water communities can be largely affected by the fish inhabiting those water systems. The introduction of benthivorous common carp has caused serious detrimental effects on the biodiversity and water quality of several lakes, rivers and ponds. Shallow waterways are affected the most (Meijer *et al*., 1999).

Some of the main ways carp activity affects water systems include the nutrient supply rates and ratios of fish excreta (Schaus & Vanni, 2000); predation (Lougheed *et al.,* 1998) and bioturbation which can be defined as the physical disturbance of the sediments caused by various activities of the fish such as feeding, walking and burrowing (Lougheed *et al.,* 1998; Moore *et al., 20*04). The common carp, which is an omnivorous benthic fish, can increase the concentration of lake water nutrients by consuming benthic organisms and excreting nutrients. It can directly consume macrophytes and indirectly by breaking or uprooting them while foraging (Lougheed *et al.,* 1998; Hinojosa-Garro & Zambrano, 2004). This reduction in macrophytes induces the wind effects (wind induced turbulence and movements) which increases sediment resuspension, which is additional to the bioturbation carp cause. These sediments will block light, which in turn affects the phytoplanktons and submerged macrophytes (Lougheed *et al.,* 1998).

The presence of carp lowered total macrophyte AFDW (ash free dry weight) biomass and total benthic macroinvertebrate species diversity by 67% (Miller & Crowl, 2006). The decrease in population of amphipods, tabanids and hirudinids, observed is probably due to the decrease in certain macrophyte species. The foraging act of carp increases the carbon resources, which in turn increases the number of chironomids and oligochaetes. Carp can affect the zooplankton and benthic macroinvertebrates biomass by acting as predators.

The direct and indirect effects of common carp can potentially initiate a ‘regime shift’ (Scheffer *et al.,* 2001). This describes the sudden shift from clear water with high macrophyte abundance to turbid water with high phytoplankton biomass, coupled with extermination of macrophytes from the system.

The fresh water ecosystem can be affected not only by biomass diversity but also by the size distribution of benthivorous fish. For example, larger carp tend to dig deeper into the sediments (Lammens & Hoogenboezem, 1991) and destroy more vegetation (Crivelli, 1983). There is an ontogenetic diet shift as carp moves from the larval stage, where it eats mainly zooplankton, to the juvenile stage, where it eats mainly benthic macroinvertebrates (Lammens & Hoogenboezem, 1991). There is a negative correlation between rate of phosphorous excretion from carp and wet weight of carp (Lamarra, 1975). Large size fish are dominant in interference competition and smaller individuals are forced into niche contraction (Persson, 1983; Persson, 1985; Polis, 1988).

Many investigations have considered the effects of carp biomass density on fresh water ecosystems but the interaction between density and size or between two adult size classes has not been investigated in detail. Driver and coworkers (2005) found that carp enclosures were more turbid, had higher nitrogen, phosphorus and chlorophyll-a concentrations, a lower pH, and more phytoplankton biomass than enclosures without carp. The highest turbidities and conductivities were observed with larger carp or higher stocking densities. The temperature 0.5 m below the water surface was lower at higher stocking densities.

The nutrient concentration in the water was also affected. Enclosures with carp had higher total phosphorus concentrations. Small carp contributed to phosphorous level via excretion (Lamarra, 1975). The feeding habits of adult carp cause bioturbation to a sediment depth of about 12 cm (Lammens & Hoogenboezem, 1991), which increases nutrient release. Therefore, having larger carp as well as higher stocking density increased phosphorous content more than small carp and low stocking density.

Carp stocking rate had a greater effect on increase in total nitrogen content than carp size. However, having a mix of small and large carp created higher total nitrogen concentrations than a single size stocking rate (Driver *et al.*, 2005).

Different methods have been used to study the effects of benthivorous fish on water systems. However, carp may have a different effect from other benthivorous fish because their feeding habits are different and higher stocking rates can be achieved (Sibbing *et al.*, 1986).

Some effects such as increase in turbidity have been consistently seen in many studies (Roberts *et al.,* 1995) but other effects on other factors such as plankton biomass have varied. Many environmental variables are very sensitive to other changes in the ecosystem (Crowder & Cooper, 1982). It is essential to simultaneously evaluate all components when studying the effects of carp. As a result, Parkos *et al.,* (2003) performed an experiment using mesocosms retaining the complexity of the natural ecosystems. Their experiment showed that the native benthic fish such as catfish did not have as strong an effect as the carp fish, which might be because of the difference in the feeding habit of carp. Carp showed most effects at high biomass compared to the effects caused at low biomass. They also suggest that the largest changes caused by carp could be in the shallow aquatic systems.

Zambrano *et al.,* (2001) suggest that all the catastrophic effects of carp on the aquatic ecosystems happen only when a critical carp density is exceeded. This means that lowering the carp biomass below the critical point could restore turbid shallow lakes to their clear state.

Some studies have found the population of waterfowl to be inversely proportional to that of carp. This might be because of the destruction of macrophytes by carp and their direct competition (Santoul & Mastrorillo. 2003).

2.3. Possible Uses of Carp: Eradication Versus Utilization

Fish can be used in several ways. The increase in the carp population in New Zealand waterways has lead to research on how to save these aquatic ecosystems from deterioration. Catching and killing the fish, then discarding them without exploring possible uses will cause extra disposal problems and thus exacerbate the environmental problem. This research aims to identify possible ways to utilize this resource, thus benefiting carp eradication from New Zealand waterways.

2.3.1. Food source

Carp were initially domesticated and commercially used as an edible fish in Asian and European countries. However, it is rarely eaten in countries into which it has been introduced. This is because carp feeds from the bottom of ponds and lakes

and the flesh tends to have an unacceptable muddy taste. However, there is potential to use this abundant source of protein and omega-3 fatty acids by developing different foods that are more acceptable and tastier.

- ***Smoked fish***

Smoking is one of the oldest methods of flavouring, cooking and preserving fish and meat before the introduction of refrigeration or freezing. The process involves two curing steps followed by a smoking step. The first curing step involves adding 4% w/w salt. During this initial stage, connective tissues break down (Doe, 1998), much of the moisture is drawn out and some of the salt soaks in. The second curing step involves adding ingredients including herbs and spices to impart the desired flavour. The cured fish is rinsed, dried and heated to thermally denature muscle proteins and inactivate any vegetative forms of microflora (Doe, 1998). The smoke is produced by burning plant materials (mostly wood). The type of wood used for smoking will imparts a specific aroma to the fish.

All types of fish can be smoked but oily fish are more suitable. There are mainly two smoking methods (Doe, 1998):

- Cold smoking, which is normally done below 30°C
- Hot smoking at about 80°C, which thermally denatures the proteins. Hot smoking is more commonly used than cold smoking.

A study on microbiological changes of hot smoked mirror carp found that the period of preservation of smoked carp depends on salt concentration and temperature (Duman *et al.*, 2007).

Fish oil is a rich source of omega-3 fatty acids, which are linked with preventing heart diseases, type 2 diabetes, and improved mental health. Smoked fish is a good source of omega-3 fatty acids (Sinclair *et al.*, 1998).

However, fish proteins can be converted to nitrosamines during smoking. An increase in nitrosamine in human diet has been linked with increased risk of gastric and oesophageal cancer (Larsson *et al.*, 2006). The omega-3 polyunsaturated fatty acid in smoked fish is extremely susceptible to light, oxygen

and heat. During the smoking process, the fatty acids are oxidised and free radicals, which are believed to cause cancer, are released (Larsson *et al.*, 2006). Smoked fish can also be a vehicle for the transmitting *Salmonella* organisms (Olitzky *et al.*, 1956). Smoking reduces the protein content and simultaneously increases the fat content of fish, decreasing nutritional value. This decrease in nutritional value is more apparent in fatty fish (Ünlüsayn *et al.*, 2001). These disadvantages mean smoked carp is not a healthy option.

- ***Canned fish***

Canning involves high heat treatment of product that has been hermetically sealed in metal or glass containers. It was a common preservation method before the introduction of refrigeration and freezing. Canning fish has the added advantage that the bones are softened, making them edible and almost undetectable.

Canning is a solution to two major drawbacks of carp: the muddy taste and the numerous bones. Research using taste panels showed that canned bighead carp was preferred over canned tuna (Thomas & Engle, 1995). The canned bighead carp had less fat than white tuna meat and about 40% of its fat is omega-3 fatty acids. Another advantage of carp is the high calcium content from its bones, which are softened and not detectable after canning.

Canning fish has some disadvantages. There are usually two heating stages during canning - the pre-treatment stage and the retorting stage. Pre-treatment involves heating the fish at about 100C°C for 1 to 8 hours (Maas-van Berkel *et al.*, 2004). This time/temperature depends on the size of the can. During this processing time, the bacteria *Clostridium botulinum* can produce spores. The vegetative cells are killed at high heat treatment but the temperature must be raised to destroy the spores because germinated spores can produce deadly botulinum toxins. Retorting involves heating at about 115°CF for 1 to 3 hours to destroy the spores (Maas-van Berkel *et al.*, 2004). These temperatures can be reached only in a pressure canner.

The twofold heating process decreases taste and vitamin content, so nutritional value of canned product is lower than fresh fish. The canning process requires expensive equipment and a lot of energy (Maas-van Berkel *et al.*, 2004), and

requires high throughput to be economically viable. Thus, canning carp is not considered an economically-viable method to utilize.

- ***Dried fish***

Fish begins to spoil soon after it is taken from the water so it needs to be preserved as soon as possible. Drying fish is one of the world's oldest preservation methods. The traditional method is to gut the fish and then hang whole fish or fish split along the spine on flakes (frame or platform for drying fish) for about three months. It is then matured for another three months indoors in a dry and airy environment. During the drying process, about 80% of the moisture is removed from the fish. The best time for the natural process is during summer (Agro Products, 2008).

Drying concentrates nutrients so dried fish is rich in proteins, vitamins, iron and calcium (Agro Products, 2008). For example, dried fish contains 62.5% protein and 2.5% total fat and has 1.21 kJ energy, 480 mg omega-3 fatty acids, 10 mg omega-6 fatty acids, 150 mg cholesterol and 7 g sodium per 100 g (Nutrition Data, 2009a). Other research (Jónsson *et al.,* 2007) also showed that dried fish in Iceland contained 80-85% protein and that these proteins were comparable to the quality of egg proteins.

There is the also the dry-salting process, which involves salting individual fish pieces and stacking them in containers. After they have been cured, the pieces are sun dried (Bala & Mondol, 2001). However, product quality can be low because crude salt accelerates lipid oxidation (Yankah *et al.*, 1996). Studies on mice (Lin *et al.*, 1986) suggest that salted dried fish can promote co-carcinogenesis (cancer development in preconditioned cells during favourable conditions).

Drying and salt drying, not only preserves fish but also impart a strong flavour, which makes it a popular sea food item worldwide. Salt drying carp can give it a good rich fish flavour, which masks the muddy taste. However, the whole process takes a long time and is difficult to produce a uniform quality. Before a dried fish product could be marketed, the acceptance of dried carp in New Zealand would need to be investigated.

- ***Fish roe***

Fish roe (eggs), a highly nutritive part of fish, is consumed in many parts of the world. It is either eaten raw or cooked and is considered a great delicacy. Top quality fish roe from freshly caught fish normally have a pleasant, non-fishy odour and taste. A single serving of roe (14 g) contains 0.08 kJ of energy, 1 g total fat, 3 g protein, 341 mg omega-3 fatty acid, 4.1 mg omega-6 fatty acid, 52 mg cholesterol and 13 mg sodium (Nutrition Data, 2009b). The popular Greek appetizer taramosalata has traditionally been made from salted and cured carp roe (Wikipedia, 2010a). An Australian company developed a process for manufacturing high value carp roe (K&C Fisheries, n.d.).

However, the high fat content of fish roe makes it very perishable, so appropriate preservation methods must be used. Some fresh water fish carry larvae of the broad fish tapeworm, which can infect humans (Salgado-Maldonado & Pineda-López, 2004). These larvae are visible to the naked eye but correct cooking or freezing can kill them.

Toxic trace elements tend to concentrate in fish, which are at the end of the food chain. A study of trace elements in six commercial New Zealand fish species reported that As, Cd, Cr, Hg, and BP concentrations were lower in the roe than in the fish muscle but Zn in barracuda roe and Cu in salmon roe were slightly higher than the acceptable levels (Bekhit *et al*., 2008). Dry salting increased trace element concentration but salting fermentation decreased the levels. Carp roe were not included in the trial and no data was found on its toxic trace element content.

Carp can spawn multiple times in a season depending on the water temperature, producing an average of 300,000 eggs per year (McCrimmon, 1968). Carp roe could be a possible product. The disadvantage is that it requires a stable fish population and the purpose of this study is to find methods to utilize carp that have been removed from the environment.

- ***Fish Burgers***

Fast food restaurants have dominated the food industry in many countries. Burgers, or specifically hamburger, are a typical fast food meal. The patties are

made of minced meat, mostly beef but occasionally other meats such as pork, chicken or turkey or meat combinations. A recent trend has been to use fish for the patties. The fish normally used are Pollock or Hoki.

An investigation showed that fish patties prepared from the fresh water fish Indian carp *Labeo rohita* were acceptable (Sehgal *et al.*, 2008). Crude protein decreased and total lipid and total soluble sugars increased when the patties were cooked. Cooking yield increased with patty weight and was higher if corn flour rather than for boiled potato was used as the extender. Patties with corn flour also had higher fat retention. The research showed that carp has potential for preparing fish patties.

To use carp for food, certain factors need to be considered. Carp flesh has an earthy or muddy flavour, which is prominent during the warmer months (Tucker, 2000). Carp can tolerate extremely polluted water and fish from these waters will not taste good. Therefore, using carp as fresh fish or for making fish patties will require a long and thorough cleaning. This may not guarantee an acceptable taste. Burger patties are prepared from fish fillets and carp has many bones, which would have to be removed. Overall, laborious processing will be required to obtain commercial quantities with an acceptable taste and texture.

- ***Fish Oil***

Fish oil is a natural product obtained by from fish in a process called rendering. The oil can be used as a dietary supplement for humans and animals and also for medicinal purposes.

Microalgae, which fish consume, produce omega-3 fatty acids. These omega-3 fatty acids then accumulate in the fish. Fresh water oily fish are one of the best sources of omega-3 essential fatty acids such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) (Schachter *et al.*, 2004).

The optimum omega-6 to omega-3 ratio is found to vary with the disease under consideration (Simopoulos, 2002). Most Western diets have too much omega-6 and insufficient omega-3 fatty acids, which can cause many diseases such as heart diseases, cancer and inflammatory and autoimmune diseases (Simopoulos, 2002).

Increasing the omega-3 essential fatty acid intake has been suggested to help reduce the risk of such diseases.

Feeding animals with either fish extracts or algae increases their DHA levels 20-fold fish, seven-fold in chicken, three to six-fold in eggs, but less than two-fold in beef (Bourre, 2005). The beneficial effect of including fish oil in animal diets can be, in turn, beneficial for humans as it provides an additional source of omega-3 fatty acids.

Silver carp are reported to have 0.19 g EPA and 0.23 g DHA per 100 g wet sample and bighead carp has 0.25 g EPA and 0.33 g DHA per 100 g (Yang *et al.*, 1994). These are similar to levels in tuna and salmon. A major advantage of carp lipid over salmon and tuna that its cholesterol level is much lower (Yang *et al.*, 1994). Carp oil has also been reported to have antitumor and antimetastatic actions (Kimura, 2002).

Carp viscera oil can be obtained by ensilaging fish wastes or from the fish meal process. There is no significant difference in composition of refined oils obtained by either of these processes (Crexi *et al.*, 2010). About 70% of the fatty acids in crude and refined carp oil are oleic, palmitic, palmitoleic, linoleic and linolenic fatty acids. Refined oil has an omega-3 to omega -6 ratio of approximately 1.05 (Crexi *et al.*, 2010).

Broiler chicken tends to develop ascites, a condition where fluid accumulates in the abdominal cavity due to heart failure. Including fish oil in broiler diets reduced the incidence of ascites (Ian, n.d.). Bone development in chickens improves if fish oil is included in the diet oil (Watkins *et al.*, 1996).

Omega-3 fatty acids are one of the most commonly-used nutritional supplements for pets. Fish oil, a rich source of omega-3 fatty acids, was first used by veterinarians to treat canine and feline allergies. Adding fish oil in pet foods also provides an anti-inflammatory effect.

- ***Pet food***

Animals also need a balanced and nutritional diet. Fish is an excellent source of protein and essential fatty acids and also contain a wide variety of vitamins and

minerals. White fish has an energy value of about 280-380 cal/lb while oily fish has an energy value of around 420-530 cal/lb (Murray & Burt, 1979).

Fish meal is a product made from fish wastes or whole fish that has been either cooked and then dried or dried directly. The process is normally called rendering. After processing, oil is extracted and the remaining material is ground to produce fish meal. Fish meal is widely used as a protein supplement in formulated feeds for pets (such as dogs and cats), monogastric animals (pigs and poultry) and for fish (aquaculture feed).

Feeding pigs, especially piglets, with fish meal and fish oil has many benefits. The essential amino acids and the polyunsaturated omega-3 fatty acids in fish meal and fish oil help reduce infections and improve the immune status of pigs and piglets (FIN, n.d.). Protein supplements, other than sow milk, may trigger inflammatory responses in piglets less than four weeks old. However, the antigenicity of fish protein is low (FIN, n.d.). This, along with its anti-inflammatory properties makes fish meal an ideal protein supplement in piglet diets. Other benefits of including fish meal in pig diets include: readily digestible; high feed intake and feed conversion efficiency; and improved growth rate. Consuming pork from pigs fed fish meal may help prevent heart disease and cancers in humans (FIN, n.d.).

The high mineral content of fish bone makes it a good natural calcium source. A study of calcium absorption concluded that calcium from fish bones was well absorbed and therefore beneficial as a supplement to pets (Malde *et al.*, 2010).

Fish hydrolysate is produced by enzymatically digesting fish to give a liquefied product that is then preserved with acids. Fish hydrolysate, differs from fish meal by containing both oil and proteins. Adding salmon protein hydrolysate and salmon meal with crushed bones to formulated feed was especially palatable in dog diets (Folador *et al.*, 2006). However, because the chemical composition and nutritional quality differs greatly, the type of fish and fish parts, should be considered when formulating the feed

Being able to incorporate fish meal and fish oil as supplements to pet diet presents an opportunity for carp products. There is also the extra benefit of its reported

high omega-3 fatty acid content. However, the response and acceptability of pets to feed that includes carp is unknown.

2.3.2. Processed non-food applications

Carp also contains components such as proteins and collagen, and enzymes such as acetyl cholinesterase. These can be extracted and purified for specific applications.

- ***Collagen***

Collagen is a group of naturally-occurring linear proteins. It is the most abundant protein in mammals, making up about 35% of whole body protein (Di Lullo *et al*., 2002) and is the main protein in connective tissue, bone, skin, joint cartilage, blood vessels, tendon and teeth. Traditional uses for collagen are as gelatin (which is collagen hydrolysed to various degrees) in foods, in photographic films and in glues (GMAP, 2005). An increasing use of collagen is for improving skin and finger nail quality. Medicinally, collagen is used in cosmetic surgeries and burn surgeries. During aging, collagen production slows and the degree of collagen cross linking increases, which causes wrinkling, joint problems and arthritis. The aging process can be slowed if the body is supplemented with additional collagen (Manning, 2005).

Collagen is traditionally extracted from pig or cow skin and bones but there has been increased interest in marine collagen sources because they are not associated with the risk of bovine spongiform encephalopathy (BSE) outbreaks. Marine collagen is also proposed as being more suitable for human skin (when used in cosmetic creams) than livestock collagen (Venugopal, 2009). Fish skin, a waste product from fish processing, is usually discarded. However, it is a valuable source of collagen. Also, fish collagen is also acceptable to the Islam religion.

Collagen has been prepared from sardine, red sea bream and Japanese sea bass fish scales by decalcifying and disaggregating the scales followed by a limited pepsin digestion (Nagai *et al.*, 2004). The collagen content (dry weight basis) was 50.9% for sardine scales, 37.5% for red sea bream scales and 41% for Japanese sea bass scales. Most of this collagen was type (alpha 1)2alpha 2.

The collagen content from Japanese sea bass, chub mackerel and bullhead shark skin were 51.4%, 49.8%, and 50.1% (dry weight basis) respectively. Collagen yields from skipjack tuna, Japanese sea-bass, ayu, yellow sea bream and horse mackerel bones were 42.3%, 40.7%, 53.6%, 40.1% and 43.5% (dry weight basis) respectively. The acid-soluble and acid-insoluble collagen yields from Japanese sea-bass fins were 5.2% and 36.4% (dry weight basis) respectively (Nagai & Suzuki, 2000). Denaturation temperature of fish collagen was lower than livestock collagen, being 25-26.5°C for collagen from fish skin, 29.5-30°C collagen from for fish bone and 28-29.1°C for collagen from fin collagen (Nagai & Suzuki, 2000).

The yield of collagen from yellowfin dorsal skin was 27.1% (Woo *et al*., 2008). This collagen had. 20.5% imino acid content and its solubility decreased when pH was above 4.0 or a salt concentration was increased to 4%. Collagen viscosity decreased as temperature increased.

The collagen type in common carp scales and bones is mainly (alpha 1)2 alpha 2 and alpha 1 alpha 2 alpha 3 is only a minor component. These two types were found in carp skin and muscle but in the swim bladder had only (alpha 1)2 alpha 2 (Kimura *et al.,* 1991). The acid soluble collagen yield by common carp skin was found to be of 41.3%, scales - 1.35% and bones - 1.06% (Duan *et al.*, 2009). The denaturation temperature of the Type I collagen was around 28°C (Duan *et al.*, 2009).

The collagen content of grass carp scales, prepared using pepsin digestions was 29.3±0.8% (Wang *et al*., 2008). The collagen was also prepared by swelling grass carp scales in water, heating the solution and then decalcifying with 8% aqueous citric acid (Chun-Mei *et al.,* 2008). After limited pepsin digestion, a collagen yield of 25.6% dry weight was obtained. This collagen was Type I collagen with a denaturation temperature between 35°C and 40°C.

These results indicate that carp can be a potential source of collagen for industrial uses. Some extraction procedures use supercritical extraction method with CO_2. However, this method requires high pressure, high capital investment, and specialized high technical skills. It probably is better to use the traditional

extraction methods unless there is an advantage to change to the more expensive process.

- ***Gelatin***

Gelatin is produced by partial hydrolysis of collagen from bones, cartilage, connective tissues and animal skins (Venugopal, 2009). This odourless, nearly tasteless, translucent and colourless material is brittle when dry but dissolves in hot water and gels when cooled. It is widely used in bakeries for jellying properties and its ability to stabilize aqueous phases. It is used to make foods such as marshmallow, cream fillings, and jellies and sweets. Gelatin is also used as a stabilizer for dairy products, in photography where sensitizing chemicals in a gelatin emulsion are coated on a glass plate, in cosmeceuticals and nutraceuticals, and in pharmaceutical industry to manufacture soft and hard capsules (GMAP, 2005). Gelatin is mainly obtained from beef bones, calf skin; hide splits, pork trimmings and from fish. Fish gelatin has the extra advantage of meeting Islamic dietary requirements.

Conventional gelatin extraction is a time consuming process involving swelling the fish skin in 50 mM acetic acid for 3 hours, extraction in distilled water for 16-18 hours at 45°C, concentrating the liquid, and then air drying the extracted gelatin (Gómez-Guillén *et al.*, 2005). Gelatin can be extracted from fish skin using a high pressure technique at 250 and 400 MPa for 10 or 20 minutes, either during the pre-treatment stage in acid at 10°C to destabilize the acid labile cross-links or during the water extraction stage at 45°C to accelerate collagen hydrolysis (Gómez-Guillén *et al.*, 2005). The high pressure process is a good alternative to the conventional process because gelatin of high gelling quality can be extracted in only few minutes. However, this method needs technically skilled workers and high capital investment.

Optimum conditions for extracting gelatin from Grass carp was established using response surface methodology (Kasankala *et al.*, 2007). These optimum conditions predicted the yield to be 19.83% and 267 g gel strength. Grass carp gelatin showed high content of imino acids and the gelling and melting points were determined to be 19.5°C and 26.8°C respectively.

Optimum conditions for extracting gelatin from silver carp predicted that the product would have a higher gel strength and yield than that of grass carp (630 g gel strength, 80.8% gelatin recovery and 6.3 cP). Silver carp skin gelatin had similar properties to gelatin from other fish that are being commercially exploited (Boran & Regenstein, 2009).

- ***Peptides***

Peptides contain two or more amino acids linked by a peptide bond between the carboxyl group of one amino acid and the amino group of the other. Fish has been used as a source of peptides for humans because they are known to inhibit the angiotensin I converting enzyme (ACE). This enzyme performs two primary functions in the human body, which result in constricting blood vessels and thus increasing the chances of high blood pressure and heart failure. Inhibiting ACE activity will relax the arterial walls. Supplementing the diet with fish peptides can help reduce blood pressure (Murray, 2009).

To use carp for such medicinal purposes, carp peptides must first be isolated and the antihypertensive properties of these peptides need to be defined. One project to isolate and define the peptide from cod fillets involved mincing the fillets, hydrolysis, centrifugation, ultrafiltration with <30kDa micro filters, followed by <10kDA and <5kDa micro filters, and fractionation using size exclusion technique. The nitrogen content and other analytical tests were done to define the peptide properties (Geirsdottir, 2009). This shows the process is laborious, requires highly-skilled workers and is expensive. Therefore, although peptides may be very useful in different ways, it probably is not profitable or commercially viable to extract peptides from carp caught in the Waikato.

- ***Acetylcholinesterase (AChE)***

Acetylcholinesterase, a neurotransmitter found in the cholinergic synapses, helps transmit nerve impulses from one neuron to the other across synapses (Bigbee *et al.*, 1999). Acetylecholinesterases are enzymes that degrade the neurotransmitter acetylcholine through a hydrolytic activity producing choline and an acetate group. These inactive molecules are then reabsorbed by the synapses and used to synthesise a new acetylcholine molecule for subsequent chemical transmissions.

If AChE is inhibited, the neuromuscular junctions are over-stimulated, leading to spasms and death caused by severe arrhythmia (Purves *et al.*, 2008).

Parkinson's disease, a degenerative disorder of the central nervous system, is characterised by tremor, rigidity, akinesia and postural instability (UMMC, 2010). People with Parkinson's have low levels of dopamine a major neurotransmitter in the brain. Dopamine depletion increases activity and production of acetylcholine, which causes the characteristic symptoms of the disease.

Acetylcholinesterase, the enzyme that breaks down acetylcholine, might be useful in treating the disease. Carp brain and muscles contain high AChE levels (Golombieski *et al.*, 2008). The AChE activity in carp is highest in serum, followed by brain, heart and trunk muscles (Szabó *et al.*, 1992). If acetylcholinesterase extracted and purified from carp muscle and brain could have various purposes in the medical field. However, extensive research is required so it is not a viable immediate process for carp from the Waikato.

- ***Bioplastics***

Plastics, which are made from petroleum-based chemicals, are considered non-biodegradable and therefore have a deleterious effect on the environment. Disposing of plastics has become a major pollution issue so there has been increased research and development on bioplastics, which are forms of plastics derived from renewable biomass sources and therefore easily biodegradable. Many vegetable proteins such as corn zein and wheat gluten and animal proteins such as milk proteins, collagen, keratin, gelatin and myofibrillar proteins can be to manufacture bioplastics (Pommet *et al.*, 2003).

Fish can be a good raw material for proteins such as collagen and keratin. Acetic acid extraction process successfully extracts collagen from fish skin, which is suitable for making biofilms (Sullivan *et al.*, 2006). Collagen can be extracted from carp bones and scales. The keratin extracted from both epithelial and mesenchymally-derived tissues of carp is similar to keratins from goldfish, zebrafish and trout fish (García *et al.*, 2005).

Fish scales may be a good source for proteins for bioplastic manufacture. Carp have cycloid scales (Wikipedia, 2010b), which grow as the fish grows. They are

arranged in an overlapping manner in a head-to-tail direction, allowing smooth flow of water over the body. These cycloid scales are thin, large, round or oval and flexible. The biopolymers in tilapia fish scales (which have cycloid scales) have been extracted and then injection-moulded into bioplastics (Suarez *et al.*, 2009). The continuing research will compare the bioplastic with commercially produced bioplastics. Using carp scales to produce bioplastics needs much further research so it is not considered a possibility for immediately utilizing waste carp from the Waikato.

2.3.3. Miscellaneous Uses

- ***Biogas***

Biogas is produced by anaerobic digestion of organic matter such as biomass, sewage, manure, municipal waste, green waste, plant material and energy crops. The gases obtained are methane and carbon dioxide.

The fish processing industry generates enormous amounts of waste, which must be treated or processed to prevent environmental pollution. One possible use could be biogas. A project to optimize anaerobic digestion of fish waste, designed in Eastern Africa (Kassuwi, 2009), found that the biogas was clean and that the digested residues could be used as organic fertilizers.

Biogas yield and digestibility of the materials can be increased if various wastes are mixed together (Mshandete *et al.*, 2004). For example, the highest methane yields from digesting sisal pulp or fish wastes separately were 0.32 m^3 CH_4/kg and 0.39 m^3 CH_4/kg volatile solids respectively for a loading of 5% total solids. However, biogas yield from co-digesting 1 part fish waste and 2 parts sisal pulp was 0.62 m^3 CH_4/kg volatile solids at 16.6% total solids. Therefore, co-digestion increased biogas yield.

Carp from the Waikato could be a good raw material for biogas production or used to increase biogas yields via co-digestion with other wastes.

- ***Biomarkers***

Biomarkers, indicators of biological state, are used in many fields such as medicine, geology, astrobiology, biochemistry, psychiatry, cell biology and

genetics. There has been increased interest in using biomarkers for environmental assessment. Water, one of the most important natural resources, is used for domestic, industrial and agricultural purposes, transport, recreation, sport and commercial fisheries, and power generation. Industrial and domestic discharges and runoff from fields treated chemical pesticides and insecticides pollute this multi-use water. The metal concentration and pesticide or insecticide content in water can be detected, measured and analysed with biomarkers.

The most commonly used biomarker is fish and various species have been used. The main principle is to measure metal content in fish tissues. *Tilapia nilotica* is sensitive to heavy metals and has been used as a biomarker to detect level of metals in Nasser Lake (Rashed, n.d.). The highest concentration of more than half the metals studied were in the fish scales.

The Common carp (*Cyprinus carpio)* is popularly used as a biomarker. A study of the environmental quality of two sites in Western Ukraine (Falfushynska & Stolyar, 2009) found that MT (metallothionein – cysteine rich proteins), TBARS (thiobarbituric acid reactive substances – low molecular end products as a result of lipid peroxidation products decomposition), and AChE in carp liver were the most sensitive biomarkers of pollution.

The AChE activity in carp brain and muscles can be used as an early biomarker of toxicity of the insecticide Diafuran (Golombieski *et al.*, 2008). The LC_{50} value for common carp, grass carp and bighead carp fingerlings exposed to Diafuran concentrations were determined by observing behavioural changes and measuring AChE levels. All fish used became lethargic or immobile when exposed to Diafuran because Diafuran inhibited AChE activity in the brain and muscle, indicating that behavioural changes or AChE activity can be used as an early biomarker of Diafuran. Pesticides and fungicides such as $CuSO_4$, paraquat (PQ), and methidathion (MD) inhibit AChE activity in fish and also influence AChE resynthesis (Szabó *et al.*, 1992). Monitoring carp brain AChE is a good diagnostic tool for chronic organophosphate and carbamate pollution (Dembélé *et al.*, 2000).

In Belgium, bioaccumulation and effects of micro pollutants in the aquatic ecosystems were assessed by exposing caged juvenile carp (*Cyprinus carpio*) to

different degrees of contamination at four different aquatic sites (Bervoets *et al.*, 2009). There was a significant relationship between metal load and changes in fish weight and condition factor, hepatosomatic index, AChE activity and a set of blood biochemical parameters. A similar study at two freshwater sites in Amsterdam (van der Oost *et al.*, 1998) using caged common carp for monitoring water pollution. The highest levels of pollutants were in carp caged for at least four weeks in the polluted sites. the hepatic phase I enzyme response was the best marker of pollution; ratio of phase I and phase II enzymes, developed as a Biotransformation index, was highest in carp caged for four to six weeks in the polluted sites.

Even though carp is useful for indicating water quality, New Zealand is trying to eradicate the species so using live fish goes does not meet the objective of eradicating this pest fish.

- ***Biofertilizer***

With increasing interest in health, people are looking for a healthier lifestyle, free of chemicals and environmental toxins. Chemical and organic fertilizers have advantages and disadvantages. Organic fertilizers are naturally-occurring fertilizers such as cottonseed meal, corn gluten meal, manure and other composts, and fish by-products. High levels of the three main nutrients for plant growth, nitrogen, phosphorus and potassium (NPK), are present in these organic fertilizers. Plants only absorb the nutrients needed for healthy growth. Nutrients in chemical fertilizers are in the form of salts. Once dissolved in water, they can evaporate, gasify or run-off. Synthetic fertilizers provide excess nitrogen, which makes plant more vulnerable to diseases and weather fluctuations, contributes to greenhouse gases, and run-off causes eutrofication in water systems (Great Pacific Bioproducts Ltd, 2010). The main benefit of organic fertilizers is increased biological activity in the soil. Microorganisms living in the soil break down organic matter, making nutrients available for plant absorption. Organic fertilizers, which can be cheaper than chemical fertilizers, encourage growth of nitrogen-fixing bacteria, allowing atmospheric nitrogen to be transformed into readily-absorbable forms for plants.

Farmers of ancient Egypt and indigenous people of North America used fish to provide fertility to their crops. In the middle of the 20th century, artificial fertilizers became prominent and it was only in the late. 20th century that the concept of organic fertilizers increased and using fish fertilizer were rediscovered. Fish fertilizers provide controlled levels of nitrogen and can increase the nitrifying bacteria in the soil. Great Pacific BioProducts have developed fertilizers from fish products (Great Pacific Bioproducts Ltd, 2010). The process uses enzymes to break down fresh fish. This hydrolysed product retains high amounts of fish proteins and other nutrients, is quickly absorbed by plants and is effective even at temperatures near to freezing. A fertilizer made by hydrolysing fish with 5% phosphoric acid has the advantage of added phosphorous and a low odour. It can be applied to soil and plants using common agricultural equipment (Robinson *et al.*, 2007).

Fish fertilizers can also be manufactured by blending fish with warm water to create an emulsion (Kristinsson & Rasco, 2000), which is then incubated with dried leaves, sawdust or brown grass clippings. The mixture is stirred daily and kept until it turns dark brown. Adding molasses helps control odour and contributes healthy microorganisms in the fish emulsion fertilizer.

These report show that fish is a promising source for manufacturing organic fertilizers. However, research on the process, odour, spread of fish disease organisms, and effect on major crops yields is needed before commercial use of carp as a bio fertilizer is accepted.

- ***Fish Hydrolysate and Silage***

Fish hydrolysate is typically made from ground fish carcasses after the fillets have been removed for human use. As the amount of by-catch increases, using whole fish to produce fish hydrolysate is becoming common. The process involves mixing minced raw material with water and ground. Enzymes can also be added to solubilise bones, scales and flesh. The liquid product putrefies rapidly and needs to be stabilized by adding acid (preferably phosphoric) to lower the pH (Baker, 1996).

Fish hydrolysate production is much less capital intensive than other processes such as fish meal because fish meal production includes cooking, pressing, drying and grinding in suitable machinery accompanied with skilled labour to obtain high quality product (Swan, 2000) while grinding and homogeneous mixing are the most essential factors for fish hydrolysate.

Fish hydrolysates are useful as fish-based fertilizers, as supplements in pet diets, for human consumption, and even in the medical field. Liquid fish hydrolysate is preferred over fish emulsion as an organic fertilizer because the cold process for making fish hydrolysate retains the naturally available fish oils, vitamins, amino acids, minerals and enzymes (Gardeningzone, 2010). The product, therefore, is a rich source of NPK for the plants. A fish hydrolysate was produced by accelerating hydrolysis using specific enzymes. This was mixed with cereals to produce an acceptable instant soup which shows its application as human food source too (Gálvez *et al.*, 1985).

Another useful property of fish protein hydrolysate is its antiproliferative activity (inhibition of cell growth especially tumour cells), making it eligible as a nutraceutical. Salmon protein hydrolysates contain significant cancer growth inhibitors (Picot *et al.*, 2006).

Fish silage is also a hydrolysed product of fish very similar to fish hydrolysate. Using enzymes to break down proteins and adding acids to decrease the pH low enough to preserve the fish hydrolysate is the main principle underlying fish silage production. Fish silage uses the proteolytic enzymes already present in the fish and added acid to accelerate the process and prevent microbial contamination. Different mineral and/or organic acids can be used.

The effect of process factors on silage process such as pH, temperature and type and amount of acid and uses of silage have to be investigated. Making silage from carp has commercial potential. It is one of the most effective ways of utilizing carp in bulk quantities with the lowest least capital investment. It requires only simple equipment, minimum infrastructure, and does not need any technically-skilled labour. It can be easily sized to fit the supply of available raw material and can use whole fish. Also, there is an increasing market for organic fertilizers due to the increased interest in organic food production. However there

is limited information on making silage from carp. Research is needed to identify the most appropriate type and amount of acid, whether combining organic and mineral acids is advantageous, ways to minimize acid usage, and the effects of other factors such as temperature and process time.

2.4. Silage

2.4.1. The process

The process to produced fermented, high-moisture fodder used to feed livestock is termed as ensiling or silaging. Silage is usually made from grass crops, with farmers ensiling pasture when there is more grass available than they need. The preserved feed is used for feeding their livestock during dry months and winter when the grass is not growing. Making hay, an alternative preservation method that involves drying surplus grass, is not always reliable in many tropical regions as forage of acceptable quality is available during wet seasons, making drying difficult. Another reason for making silage is that livestock may prefer it to hay as it is moist and thus more palatable. It also retains a much larger proportion of nutrients than hay (Living Countryside, 1999).

The ensiling process involves wilting cut grass to reduce moisture content to 60-70%, which is the optimum moisture level for fermentation, then chopping it into smaller pieces and compacting it to exclude all possible oxygen. Any oxygen present allows aerobic bacteria to consume carbohydrates, thus reducing nutrient content of the ensilage (Schroeder, 2004). The compacted piles are sealed with plastic sheets and left to ferment.

The initial anaerobic bacteria during ensiling are the acetic acid producing bacteria, which ferment carbohydrates and produce acetic acid. When the pH falls below 5.0, the acetic acid producing bacteria are inhibited and lactic acid producing bacteria dominate. The lactic acid fermentation is the longest phase of the ensiling process and drops the pH further until all bacteria are inhibited (Schroeder, 2004). When pH reaches pH 4, sugars stop breaking down and the silage is preserved. Acetic and lactic acids are the most desirable products. "Quality silage is achieved when lactic acid is the predominant acid produced, as

it is the most efficient fermentation acid and will drop the pH of the silage the fastest. The faster the fermentation is completed, the more nutrients will be retained in the silage" (Schroeder, 2004). Nowadays, specific microorganisms are inoculated to bulk silage to increase fermentation rate or improve silage quality. If the moisture content during ensiling is above 70 percent, clostridia bacteria grow in the silage, producing butyric acid instead of lactic acid and making the silage sour (Schroeder, 2004). If the pH does not decrease sufficiently, by-products such as ammonia are produced. These by-products can decrease the palatability to cattle (The University of Waikato, 2005).

Another promising raw material for making silage is fish. The idea of fish silage originated in Sweden in the 1930s and Denmark started commercial production about ten years later. Fish silage is a liquid product made from whole fish or parts of fish that are liquefied by the action of fish enzymes and added acids (Tatterson & Windsor, 2006). The enzymes break the fish proteins into smaller soluble units while the acid increases the process and prevents bacterial spoilage.

2.4.2. Fish silage

The fish silage process is cheap and simple. The raw material, which can be either whole fish or fish waste, is minced, and then organic acid, mineral acid or a carbohydrate source is added. Fish silage made by adding mineral or organic acid is called acid fish silage whereas silage produced with a carbohydrate source and anaerobic conditions is called fermented (or biological) fish silage (Pérez, 1995).

Acid silage

Acid ensilage of fish offal was invented in the 1920s (TDRI, 1982). Fish by-catch or fish waste is minced and mineral and/or organic acid added (Fig. 2.3). The material must be mixed thoroughly to avoid untreated material, which will putrefy. The pH of should be pH 4 or lower to prevent bacterial spoilage (Gildberg, 1993). Therefore, it is important that silage production tanks are acid resistant. Occasional stirring after the silage process has started helps to ensure uniformity. If a fatty fish is used for making silage, the mixture will be more homogenous but the oil present can deteriorate rapidly.

Fish silage with the appropriate amount of acid can be stored for up to two years without putrefaction. During storage it becomes smoother and develops a pleasant malty odour (Tatterson & Windsor, 2006).

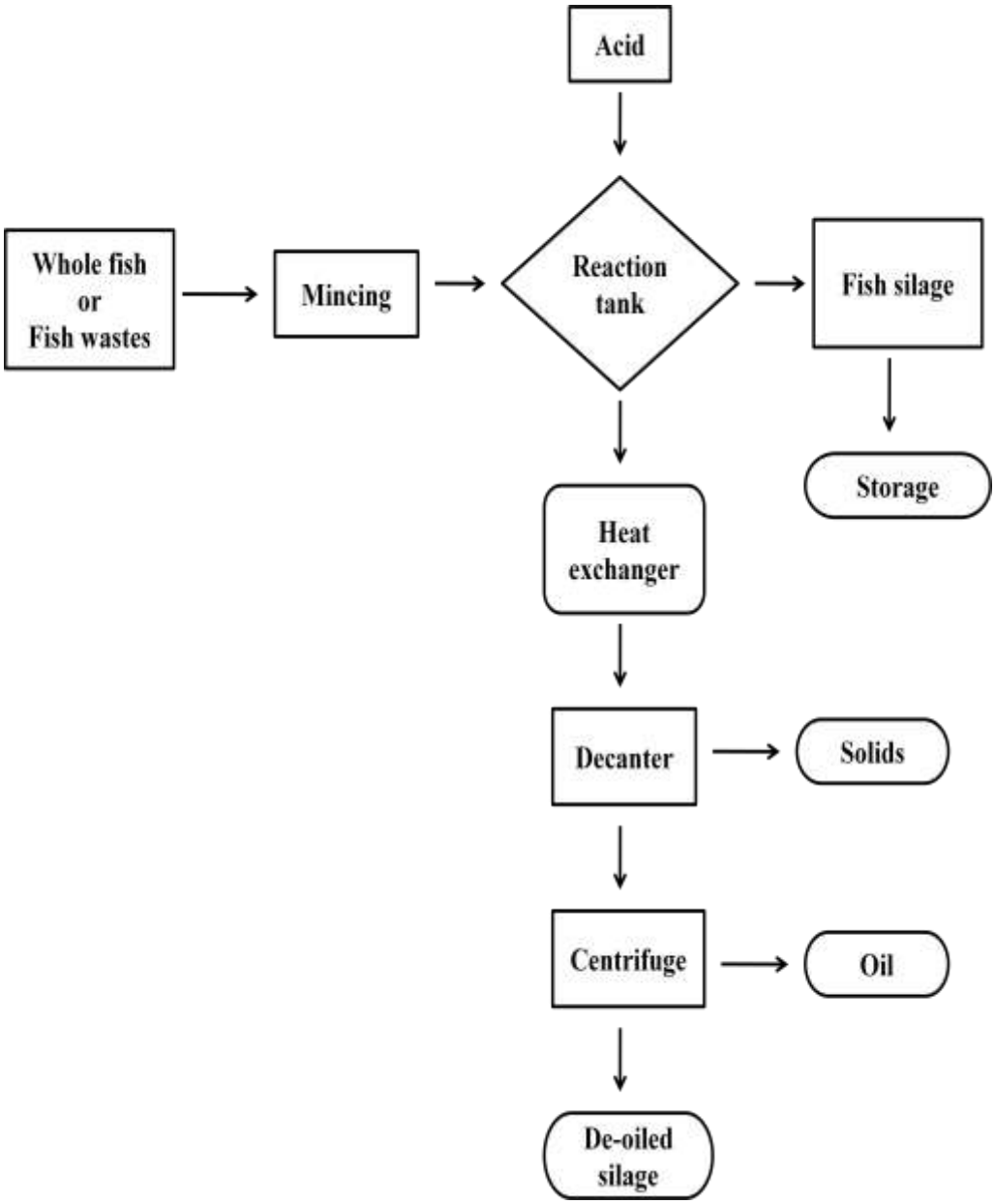

Figure 2.3: Silage Production Process

Factors affecting liquefaction rate

Research has shown that most species of fish can be used for making silage. Fatty fish and fresh fish liquefy more quickly than white fish offal and stale fish and sharks and rays tend to be difficult to liquefy (Tatterson & Windsor, 2006).

The process can be increased by heating the mixture. Liquefaction is slow at lower temperatures so the temperature should be at least 20°C (TDRI, 1982). Higher temperatures can inactivate the enzymes responsible for liquefaction, but the materials liquefy rapidly at 40°C (TDRI, 1982). Raw materials stored at 2ºC and 23ºC have to be kept for long periods, sometimes for up to a year, to produce a silage product (Tatterson & Windsor, 2006). Keeping for longer times causes oils in the silage to undergo rapid deterioration and soluble nitrogen content to increase.

Acids are added to acid silage to increase activity of the enzymes responsible for liquefaction. The acidic pH also prevents bacterial spoilage. Acids commonly used include mineral acids such as sulphuric acid and hydrochloric acid, and organic acids such as formic acid and propionic acid (Pérez, 1995). These acids can be used singly or as a combination of mineral and organic acids. The low pH caused by adding mineral acids can cause corrosion and so the pH is raise to 5 by adding calcium carbonate (Alvarez, 1972) or calcium hydroxide (Pérez, 1995). Preservation with organic acids can be achieved at slightly higher pH levels so less acid is required and neutralization is not required. Formic acid has some bacteriostatic action and is a good option but more expensive than mineral acids. The pH when adding formic acid should be 3.6-4 (Tatterson & Windsor, 2006).

Fermented silage

Although the principle for making fermented silage is the same as for acid silage, the additive is a carbohydrate source (Pérez, 1995). The silage is preserved by the acidity caused by lactic acid producing bacteria. Raw material is minced and placed in non-metallic containers then mixed with a carbohydrate source such as molasses, sweet potato or cassava. The process is maintained under anaerobic conditions.

2.4.3. Studies on Fish Silage

A comparative study (Vidotti *et al.*, 2003) investigated the effect of different raw materials on the amino acid composition of fermented fish silage and acid fish silage made from commercial marine fish wastes, commercial fresh water fish wastes and tilapia filleting residue. Fermented silage was produced by adding 50 g/kg *Lactobacillus plantarum* and 150 g/kg sugar cane molasses. Acid silage was produced by adding 20 ml/kg formic acid and 20 ml/kg sulphuric acid.

The silages contained less crude protein than the raw material (Table 2.1) but acid silage has higher crude protein content than fermented silage.

Table 2.1: Effect of raw material and process on crude protein (g/kg dry matter) in the final silage (from (Vidotti, *et al.*, 2003))

Marine fish waste			Fresh water fish waste			Tilapia filleting residue		
Raw	Fermented silage	Acid silage	Raw	Fermented silage	Acid silage	Raw	Fermented silage	Acid silage
776.7	596.1	699.1	496.2	420.9	443.8	429.9	358.4	395.9

The amino acid analysis of the raw materials and silages showed that histidine, threonine and serine levels increased in both acid and fermented silages and valine, isoleucine, and leucine levels decreased. The nutritive value of the silages, calculated using the FAO/WHO chemical score for amino acid content and Nile Tilapia nutrient requirements showed that all silages had a suitable amino acid profile and could be used for a balanced fish diet (Vidotti *et al.*, 2003).

The lipid content of acid silage (AS), biological silage (BS) and enzymatic silage (ES) made from Nile tilapia was 12.45, 12.25 and 12.17 g/100 g dry matter respectively (Borghesi *et al.*, 2008). The fatty acids were predominantly unsaturated ones such as oleic acid. The saturated fatty acids stearic and palmitic acids were also present.

A comparative study (Penedo *et al.*, 1986) of by-catch and fish wastes added 35, 40 and 50 ml of commercial sulphuric acid to 700 ml of water, and then mixed

this with 1 kg of by-catch or fish wastes. After three days, an equal amount (volume) of molasses was added. The pH of samples with the weakest acid was 4.97 and 5 (by-catch and fish wastes respectively) whereas those with the strongest acid had a pH of 4.28 and 4.3 respectively. Samples with the weakest acid could be preserved for three days, those with slightly more acid could be kept for 15 days, and the samples with the strongest acid could be preserved and stored up to one month. Silage made from by-catch contained more than 9 percent extraneous, calcareous matter, mostly in the form of large crustaceans that could not be easily attacked by the acid. This caused putrefaction, making by-catch inferior to fish wastes as a silage material.

There are many studies on optimising the amount of acid to preserve fish silage. One study used different amounts of 50% v/v sulphuric acid for ensiling unchopped fish. Adding 60 ml of 50% sulphuric acid per 1 kg of fresh fish waste was found to be optimum (Alvarez, 1972). A further study with 50% v/v sulphuric acid (Cervantes, 1979) showed that only half the acid (i.e. 30 ml/kg fish) was needed to preserve whole by-catch that had been minced

Organic acids can preserve fish silage at a higher pH, eliminating the need to neutralize the product before it is used. A study added 3.5% (v/w) of 85% v/v formic acid or a 1:1 mixture of formic and propionic acid to fish wastes that had been chopped to 4 mm to make fish silage (Green *et al.*, 1983; Rattagool *et al.*, 1980; Wiseman *et al.*, 1982).

If a combination of organic and mineral acids are used, adding mineral acid to obtain pH 3 plus 0.5% formic acid produces a good quality fish silage. If formic acid is to be used alone, 3% formic acid by volume to weight of fish was suitable in most situations (TDRI, 1982). Kompiang (1981) recommends a mixture of 1:1 formic-propionic acids, added at 3% volume/weight of biomass to produce silage that is stable and has an acidified aroma (Kompiang, 1981).

Other process conditions influence making a good quality silage. A raw material particle size of 3-4 mm diameter and a temperature of at least 20°C produces good silage (TDRI, 1982). Stirring is also important to ensure there are no regions for putrifaction to occur. One study stirred the mixture for three minutes, three times a day for five days to provide uniform mixing (Alvarez, 1972).

Any oil liberated as the fish liquefies can deteriorate rapidly and should be removed. One way to recover the oil is to increase the temperature to 65-70°C and then centrifuge the mixture (TDRI, 1982).

Liquid silage is very difficult to transport so more recent studies have investigated how to produce dry silage. This can be made by mixing liquid silage with a powdered or granular carbohydrate source such as rice or bran. The added carbohydrate absorbs some of the moisture and the paste can be sun dried into a product with nitrogenous protein as well as an energy source from the added carbohydrate (TDRI, 1982).

Another way for compacting silage for easier handling is to increasing the viscosity. This can be achieved by adding formaldehyde, which, also reduces the rate ammonia is released from the silage protein. Adding 5 ml of 40% v/v formalin solution per kg of acid-preserved silage was found to be the optimum for improving protein value of silage for feeding to ruminants and for increasing its viscosity (Husain & Offer, 1986). Adding higher amounts of formaldehyde produced a rubber-like product that was unsuitable for animal feeding.

There have been many studies on the type and amount of carbohydrate to use, along with the effect of other factors such as temperature. The bacterium *Lactobacillus plantarum* is mainly used for the fermentation (Hassan & Heath, 1987). Total amino acids increased after fermentation. Storing fermented silage at 37°C for 35 days reduced moisture content, increased fat content and increased the content all amino acids. The changes in fat and amino acid content are partly due to the reduced moisture. Lipolysis will increase free fatty acid content so oil extracted from the silage will have lower quality (Hassan & Heath, 1987).

The different studies have indicated that different proportions of different additives can be used to make fish silage. Fermented silage can be prepared by adding 5% bacterial culture to a 60% by-catch, 30% ground maize and 5% molasses and used for feeding pigs (Tibbetts *et al.*, 1981).

Rapid liquefaction can occur if the temperature is at least. 20°C and there is periodic agitation (Green *et al.*, 1983). Adding 20-30% molasses helps start the fermentation readily (Domínguez, 1988).

A trial in Morocco involved mixing (50:50 w/w) molasses and drained ground fish wastes (Hassan, 1994). This mixture was left uncovered and stirred daily. It took a long time to produce a stable product of pH 4.5. Minerals and vitamins (1%) and about ground straw (20%) were added and 7 to 8-kg blocks were dried in the sun then used as solid feed for sheep, goats, horses and camels (Hassan, 1994).

Perez (1988) first proposed in a 1986 FAO Expert Consultation that "whole" fish wastes can be preserved directly in cane molasses. Later, trials showed that the raw material should be minced before it is mixed with equal amounts (by weight) of molasses. In the initial stages of the silage process, raw tissue is dehydrated due to the osmotic pressure caused by the molasses. The acidic fermentation that is also occurring tends to preserve the material (Dominguez, 1988; Hassan 1994). If the raw material is whole, it should be completely submerged in molasses and the mixture should be stirred twice daily, for five to seven days.

Fish wastes from the fish processing industry have also been used for making biological silage. Trials to investigate the effect of mincing, molasses concentration, process temperature, *Lactobacillus plantarum* inoculation, and using tropical fruits as a source of proteolytic enzymes (Bello & Brito, 1994) showed that the fish must be reduced to very small particle size, molasses concentration should be at least 15%, and process temperature should be around 40^{o}C. A microbial inoculation was required and at least 10% percent tropical fruit wastes should be used. A stable product was produced after 90 days at room temperature. This research supported using tropical fruits as a silage additive. Another study on adding tropical fruit wastes (Reyes *et al.*, 1991) mixed fish 85:15 with either sterile molasses or tropical fruit wastes or mixed fish with molasses and fruit wastes in the proportion 72.5:12.5:15. The material was kept at various temperatures between 35 and 75°C for 17 days. Adding fruit wastes significantly increased silage liquefaction, which was most rapid using 15% fruit wastes at 55°C without any molasses. However, to produce stable silage, molasses was needed.

Kiwi fruit is a sub-tropical fruit grown in New Zealand. It contains large amounts of a cystein protease actinidin (Mostafaie *et al.*, 2007). The highest actinidin

activity is in the flesh, followed by zones around the seeds and core (Préstamo, 1993). This enzyme, which also called the meat tenderizing protease (Mostafaie *et al.*, 2007), has been widely used for tenderizing meat. For example, treating beef slices with actinidin at 37°C for 2 hours increased tenderization substantially (Aminlari *et al.*, 2009). It is possible that kiwi fruit could be used to make fish silage.

2.4.4. Uses for fish silage

- ***Animal feed***

Fish meal as a high nutritional value and is a protein source for livestock feeds. It can be used in animal feeds in a similar way to fish meal. Fish silage contains the amino acids lysine, threonine and sulphur-containing amino acids present in fish meal (Whittemore & Taylor, 1976). However, fish meal has 65% protein whilst silage has only 15% (Tatterson & Windsor, 2006). Therefore, four times as much silage is required to obtain the same amount of protein. Despite this, fish silage has many advantages that make it a suitable substitute for fish meal. Unlike the fish meal process, fish silage processing equipment cost is low, technical and skilled labour is not required, and there is no odour problem. The digestible energy and nitrogen content of a diet with fish silage is higher than one made with fish meal (Whittemore & Taylor, 1976). Silage can also be advantageous in regions that do not have fish meal plants (e.g. Greece) or sufficient raw material to establish a processing plant (Balios, 2003). However, transporting fish silage is more expensive because it is four or five times as bulky as fish meal.

Fish silage has been used in pig farming. Trials have shown that pigs grow as fast on silage as on meal and that meat quality is good (Tatterson & Windsor, 2006). Fish silage has also been used in feed for cows and poultry, and has been used in aquaculture feed (Tatterson & Windsor, 2006). The Cuban Ministry of Agriculture has set up and run fish silage factories to produce a protein-rich feed for ruminants (MINAG, 1975). By-catch and/or fish wastes are placed in concrete tanks and completely covered with water (at least 2.5 cm above the material). Concentrated commercial sulphuric acid is then added at 8 to 9% by weight or 5% by volume (i.e. 50 litres or 90 kg of acid per tonne raw material). After the

material liquefies, the oil is skimmed from the surface and used in rations for calves. The final product is neutralized to pH 6 with calcium hydroxide or carbonate and then mixed with an equal volume of molasses and used in cattle feedlot operations or mixed 1:1 with wheat bran and used for feeding calves (MINAG, 1975).

A silage made by adding 3.5% (v/w) of 85% formic acid to minced fish has been included in pig diets. Pigs fed silage has higher daily live weight gains than pigs fed a fish meal diet and the carcasses had acceptable eating qualities (Machin *et al.*, 1982). A study on the appropriate proportion of silage to use in pig diets found that adding 100 g fish silage per kg diet dry matter gave very good performance (Green *et al.*, 1988).

Fish silage can be included in poultry diets. Including 5% fish silage in broiler diets gives the best biological response and acceptable meat quality and acceptance (Rodríguez *et al.*, 1990). It can also give higher egg production, thicker egg shells and better feed utilization (Balios, 2003). Another study on adding fish silage and additional fish fat in chicks' diet showed that fatty acid content increased in abdominal fat and breast meat and that adding fish fat in the diet decreased blood plasma, Vitamin E and ceruloplasmin level. However, high levels of fish fat caused off-odour and off-taste in thigh meat (Kjos *et al.*, 2000).

Biological fish silage was made by mixing fish with molasses, papaya and pineapple wastes, inoculating the mix with *Lactobacillus plantarum* and storing it for seven days before drying it. Trials showed that chickens preferred diets with up to 50% fish silage over the diets with no fish silage (Bello & Fernández, 1995).

Fish silage can be used in aquaculture feeds. Including 30-35% fish silage in fish diets (Enke *et al.*, 2009) increased body weight gain, total body length, final body weight, and specific growth rate. Better production performance without any adverse effects on survival and water quality was also achieved.

The apparent digestibility coefficient of crude protein of acid silage, biological silage and enzymatic silage made from Nile Tilapia was 92.0%, 89.1% and 93.7% respectively and the average apparent digestibility of the amino acids was 91.8%, 90.8% and 94.6% respectively (Borghesi *et al.*, 2008).

Methane produced by methanogenic bacteria that live in the digestive systems of ruminants contributes to green house gases. Nearly one third of New Zealand's total green house gas emission is produced by farm animals Methane production can be reduced by including fish silage in animal feed (United Fisheries, 2010). Fish silage contains 10% fish oil rich in omega-3 fatty acids. Thus, fish silage has the dual benefits from the omega-3 fatty acid content on improved meat quality as well as helping reduce methane emission.

- ***Organic fertilizer***

The demand for organic fertilizers has risen with increased awareness of the deleterious environmental effects of chemical fertilizers. Studies on the effect of fish silage, fish bone meal, seaweed extracts and chemical fertilizer on various crops showed that yields and size of plants receiving organic amendments were comparable to those using chemical fertilizer in most seasons (Blatt, 1991).

Synnes and Opstad (1995) applied salmon silage (25.3% dry matter, 19 kg N per ton) and mineral fertilizers on Italian Ryegrass to compare organic and chemical fertilizer performance difference and found that applying. 20 tons salmon silage per hectare gave approximately the same yield (dry matter) as the fields applied with NPK fertilizers (Synnes & Opstad, 1995).

- ***Oil***

Fish oil is an important source of polyunsaturated omega-3 fatty acids, which are beneficial to human health. Oil characteristics can be affected by impurities. High-quality oil can be recovered from ensiled mackerel and sprat. During liquefaction, the free fatty acids are released. Oil recovered by centrifugation contains increased free fatty acids levels. Acid hydrolysis also releases haemoglobin hemin (hemin - iron-containing porphyrin) which can pigment the oil. Adding 2% hydrogen peroxide can reduce the free fatty acid and pigmentation (Reece, 2006).

Natural enzymes in a silage prepared from salmon fish viscera with 2% formic acid also hydrolysed some of the triglycerides, which increases feed value. The resultant silage had 18% saturated fatty acids, 42% monounsaturated fatty acids, 23% omega-3 fatty acids, 10% omega-6 fatty acids and 6% EPA (NIF, n.d).

A silage made by adding 10% glacial acetic acid to fish and keeping it 50^{o}C for 15 days was centrifuged to give fish meal and oil plus acid. This crude oil can be refined for dietary use. Winterizing oil can increase the polyunsaturated fatty acid levels (EPA, DHA) by 8.5% t 62% and reduce saturated fatty acid content by 12.5% (Crexi *et al.*, 2007). Fish viscera have a total lipid content of 19-21%. After ensiling, 85% of this can be recovered (Rai *et al.*, 2010).

2.5. Aims of the research

The many studies on fish silage and its uses indicate that it is a useful. It can be made easily and cheaply. The aim of the research was to find ways to use koi carp that were collected in the eradiation process. Processes and products based on long-term, sustained source of raw material were not suitable. Nor were processes/products that had not been fully researched or for which there were not immediate markets without further research. It was decided that using whole minced fish to make silage met the criteria of low-cost and low technology process with a product that had an end market. However, further research is needed on to identify the optimum processing conditions that minimized process costs. Some experimental studies on the effect of pH, temperature, acids and kiwi fruit as proteolytic source on fish silage follows in the upcoming sections of the thesis.

3: MATERIALS AND METHODS

This chapter describes the trials done to investigate the effect of different processing conditions on the rate of silage production. Silage production was assessed by measuring the change in soluble solids content. A series of preliminary trials investigated the effect of stirring, temperature and pH. After identifying the most appropriate conditions, a further set of trials were done to investigate the effect of different acids (mineral and organic) used singly or in combination and the effect of adding kiwifruit as a silage additive.

3.1. Materials

3.1.1. Fish

Koi carp from Lake Waikare, which was captured with the help of personal from the Biological Science Department of Waikato University, were used in the study. Lake Waikare is a shallow lake (1.5-1.8 m deep) in the lower Waikato catchment system. The lake has no large submerged aquatic plants and has low water quality (EW, 1999-2010). Since 1993, there have been significant increases in total nitrogen and total phosphorus, and increased chlorophyll a. The levels of suspended sediment are very high and water quality is highly variable.

The fish were caught using electrofishing, which involves the emission of electric pulses at a frequency of 60 pulses per second of 2-4 amp root mean squares. Two adjustable 1-m long anodes create the fishing field about 4 m wide. The electric pulses stuns all the fish in this field, making them float to the surface. Only the koi carp were selectively captured (Hicks *et al.*, 2005).

The fish had an average length of 30 cm and weighed about 1.3 kg. After the fish had been caught, they were kept in boxes with ice, transported to the Waikato University and stored in a freezer at -21^{o}C.

To prepare the fish for the trials, whole frozen fish were chopped into 7.5 cm X 5 cm pieces with a knife and axe, then ground through a 5-mm holeplate in a commercial mincer. The minced fish was mixed and packed into approximately

1-kg lots in plastic bags, then refrozen and stored at -21°C for a maximum of 8 weeks. When required, the minced fish was taken out of the freezer, thawed at room temperature, then divided into required amounts and sealed in plastic bags for trials.

3.1.2. Chemicals and additives

Acids: Technical /analytical grade acids from Ajax Finechem Pty Ltd, were used.

Sulphuric acid: 98%, 18.4 M, 36.8 N

Hydrochloric acid: 36%, 18.4 M, 11.65 N

Nitric acid: 70 %, 15.8 M, 15.8 N

Formic acid: 99 %, 26.5 M, 26.5 N

Acetic acid: 99.5%, 17.4 M, 17.4 N

Citric acid: 50 %, 5.47 M, 5.47 N

Kiwifruit: Ripe green and golden kiwifruit were obtained from a local fruit shop. Five whole (skin, pulp and seeds) green kiwifruit (GKC or green kiwifruit crude) and five golden kiwifruit (goldKC or golden kiwifruit crude) were blended in a household blender (750 watts) and put into separate labelled containers. The flesh (without seeds and skin) of a further five green kiwifruit and five golden kiwifruit was spooned out, pulped in a blender and transferred to containers labelled GKF (green kiwifruit flesh) and goldKF (golden kiwifruit flesh). These kiwifruit were all used fresh for the respective trials.

3.1.3. Equipment

Knives and an axe were used for chopping the fish. Samples were stored in various sized re-sealable plastic bags. The small-scale trials were done by putting aliquots of fish into 27 mm X 18 mm re-sealable plastic bags stored in plastic boxes with lids. Material was stirred with wooden spoons. Various size watch glasses, porcelain dishes, measuring cylinders, No 1 grade Whatman filter paper, and eye droppers were used for the analytical tests.

3.1.4. Analytical methods

Dry matter: Accurately weighed samples were placed on watch glasses or porcelain dishes and dried in either an 80°C or 100^{o}C oven for 24 hours. The residual material was expressed as dry matter per 100 g of original sample.

pH: The pH was measured using a glass electrode.

Temperature: The temperature was measured using thermocouple probes with digital display.

3.2 Trials

Preliminary trials

Preliminary trials explored the effect of stirring on rate, temperature and pH (Fig. 3.1).

- Stirring conditions, ensiling time, temperature and pH

Batches of 500 grams of minced thawed carp were put into a re-sealable bags and then 10 mL 18.4 M sulphuric acid was added and mixed with a wooden spoon. The pH and temperature were measured before the bag was sealed and stored at room temperature (approx, 20^{o}C) for a maximum of 10 days.

Sample A: On each of the first 10 days, the bag was opened and the contents were mixed with a wooden spoon. The pH and temperature were measured daily. No further acid (to adjust pH) was used during this trial.

Sample B: This sample was kept closed for 10 days. Each day, the contents were mixed by squeezing the bag. The pH and temperature were not measured. The 'texture' of the sample was assessed visually and recorded.

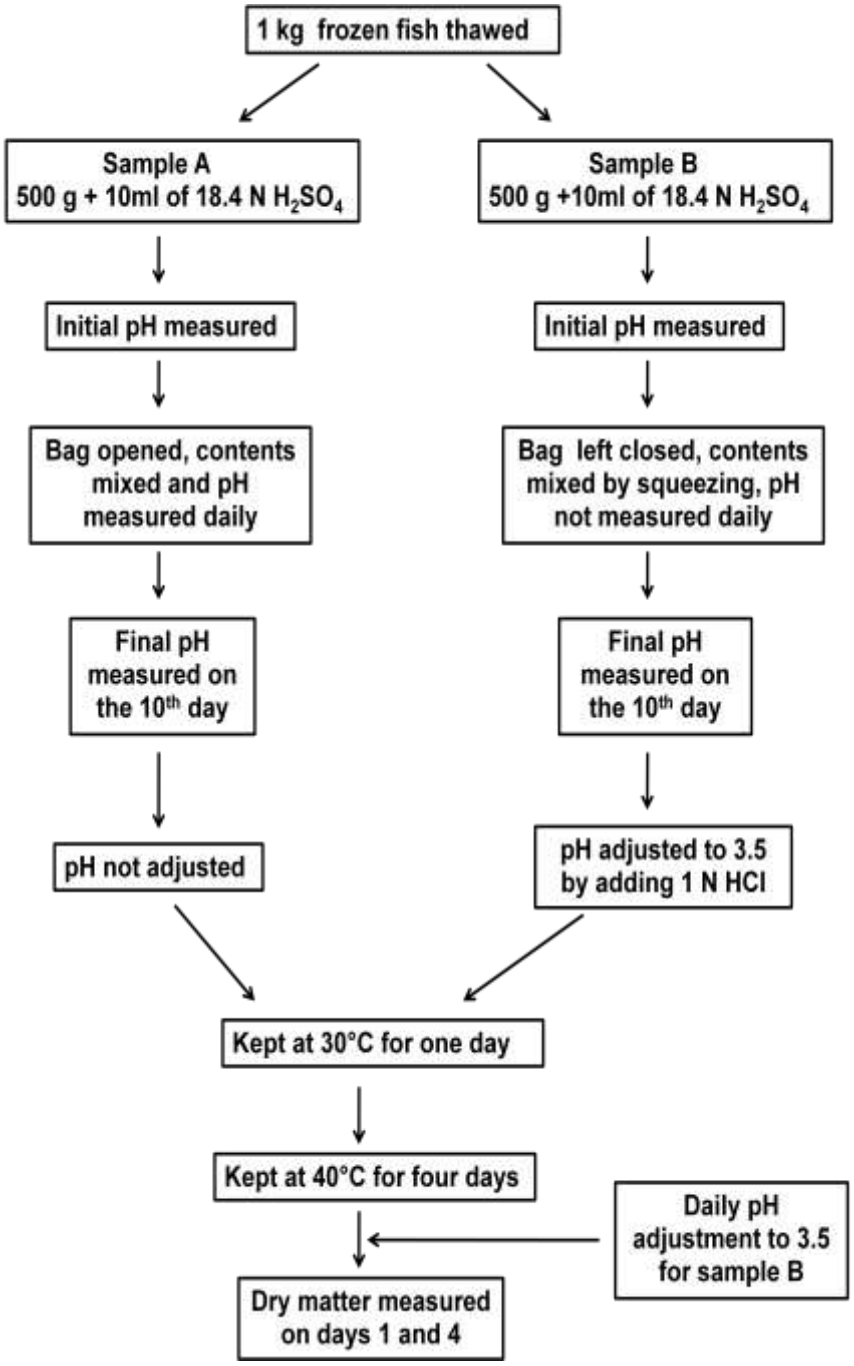

Figure 3.1: Steps in the preliminary trial on effect of stirring conditions, ensiling time, temperature and pH

After 10 days, samples A and B were kept at 30°C for one day before being transferred to a 40°C oven and kept for a further four days until the samples had liquefied completely. In this part of the trial, the pH of sample B was daily adjusted to 3.5 using 1.5 ml 18.4 M sulphuric acid. The pH of sample A was not adjusted.

On days 12 and 15 of the 15-day trial, a well mixed sample (50 g from 40°C) was taken from Samples A and B and used to measure the dry matter.

- Temperature

Samples (100 g) of thawed minced fish were put into six re-sealable plastic bags and 22 ml 1N HCl was added to each bag to bring the pH to 3.5. The samples were mixed well using a wooden spoon and then the bags were sealed. The bags were kept in ovens at 25°C, 30°C, 35°C, 40°C and 45°C for four days. The control sample (with the same amount of acid) was kept at room temperature (19°C). The pH was monitored daily and 1N HCl added to maintain the pH at 3.5.

After four days, the dry matter of 50 g sub-samples was determined.

- pH

Six 100 g samples of thawed minced fish were put into re-sealable plastic bags (Fig. 3.2). Aliquots of 1 N HCl (50, 33, 22, 17, 6 and 2 ml respectively) were added to adjust the pH to 2.0, 3.0, 3.5, 4.0, 5.0 and 6.0. The control had no added acid.

All the samples were then kept at 40°C for two days. The pH was measured after one day and acid added to maintain the set pH. After two days, all samples were removed from the oven and a 50 g sample taken. This was filtered through No 1 filter paper (Whatman) overnight at room temperature. The filtrate and solids on the filter paper were dried for 24 h in an 80°C oven. The dry weight of the filtrate and solids was expressed as g per 100-g of original sample.

Another 50-g sample from this trial was centrifuged at 4000 rpm for 20 minutes. A visual observation of the layers obtained was noted. The liquid and solid phase was separated and their dry matter was expressed in g per 100 g of original sample.

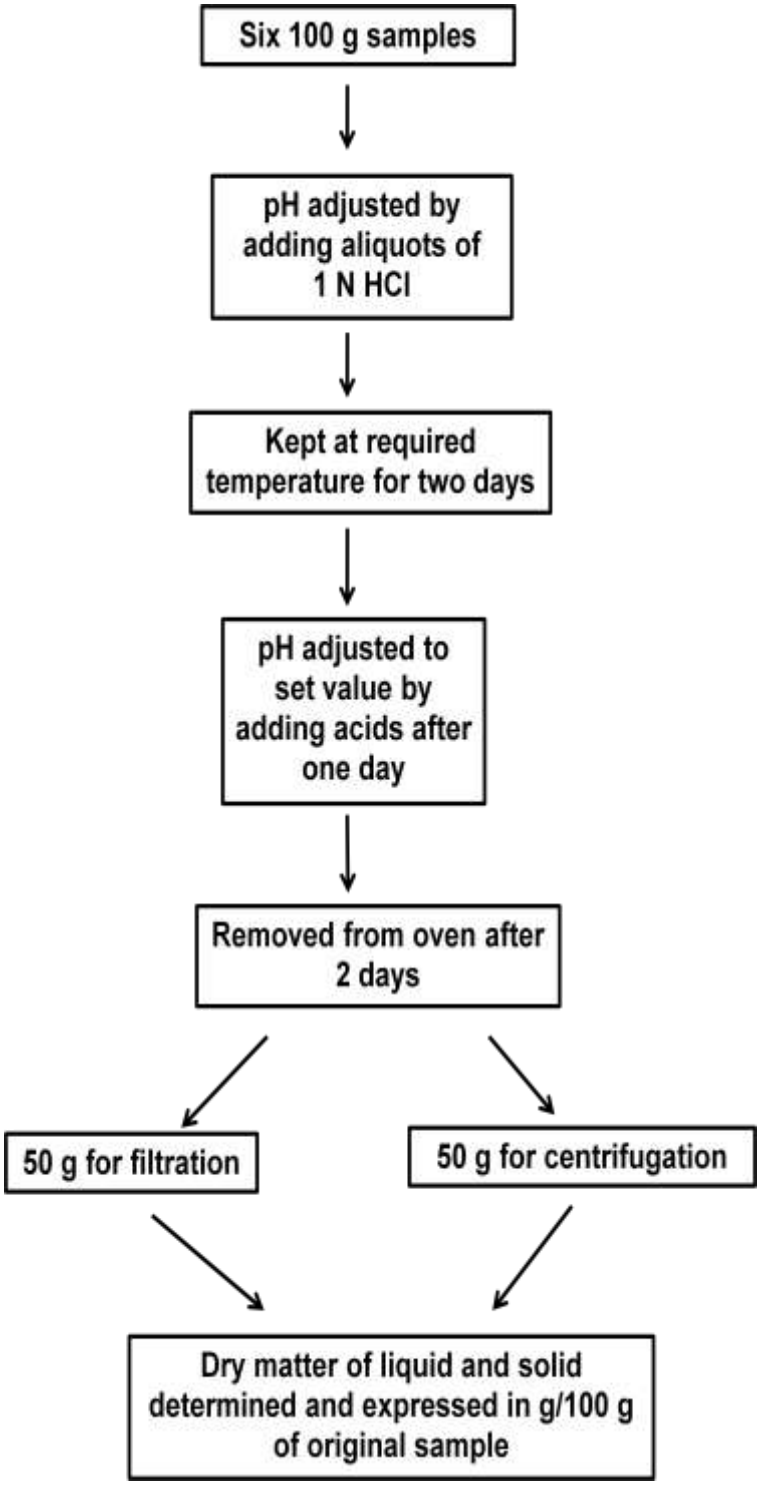

Figure 3.2: Steps involved in the preliminary trial studying effect of pH

Main experiments

General methodology: Minced fish was thawed and divided into 100-g lots and put in re-sealable plastic bags. The required amount of acid was added and the samples mixed well using a wooden spoon. Samples were kept in a 40°C oven for four days. The control sample was kept at the same temperature but with no additives (no acid, no kiwifruit). The pH was measured after 1.5, 3 and 4 days and acid added to bring the pH to the set value. Samples were mixed with a wooden spoon before being returned to the oven (Fig. 3.3).

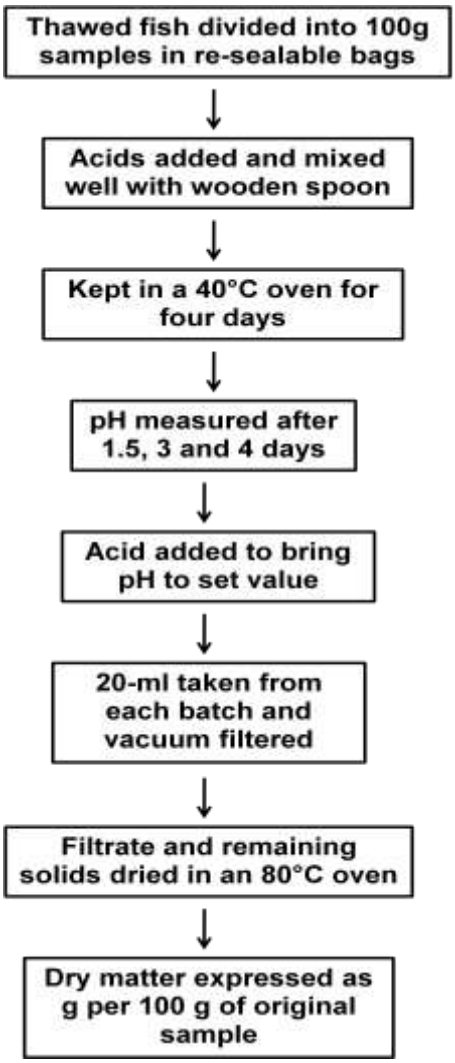

Figure 3.3: General methodology for trials

At 1.5, 3 and 4 days, a 20-ml was taken from each batch and filtered by vacuum. Samples of the filtrate and remaining solids were dried in an 80°C oven. Dry

matter was expressed as g per 100 g of original sample. The pH of the remaining samples was measured, readjusted to the original value and properly mixed with a wooden spoon before keeping them in the oven again.

All measurements were done in triplicate. The main experiment was repeated three times and analytical data were averaged.

- Type of acid

The 100-g samples in re-sealable plastic bags were mixed with 0.7 ml of concentrated sulphuric acid, 17 ml of 1N hydrochloric acid, 6 ml acetic acid, 2 ml 99% formic acid, 5 ml 50% citric acid and about 2.5 ml nitric acid to adjust the pH to 4.0. The samples were kept in an oven at 40 °C for four days after which the dry matter of filtrate and solids was determined after 1.5, 3 and 4 days.

- Combining acids

Mixtures (50:50 v/v) of various acids were prepared and added to 100-g samples to give a pH 4. The following acids were used:

sulphuric acid: acetic acid →1:1; 18.4 M: 17.4 M
sulphuric acid: citric acid → 1:1; 18.4 M: 5.47 M
sulphuric acid: formic acid →1:1: 18.4 M: 26.5 M
hydrochloric acid: acetic acid →1:1; 18.4 M: 17.4 M
hydrochloric acid: citric acid →1:1; 18.4 M: 5.47 M
hydrochloric acid: formic acid →1:1; 18.4 M: 26.5 M

After adding the acid combination to the samples in re-sealable plastic bags, the samples were kept in a 40^{o}C oven for up to 4 days. The dry matter of samples taken at 1.5, 3 and 4 days was determined.

- Adding kiwifruit

Green Kiwi flesh (GKF), golden Kiwi flesh (goldKF), green Kiwi crude (GKC), and golden Kiwi crude (goldKC) were used as a source of endogenous proteolytic enzymes. The prepared samples were stored in a 40^{o}C oven for up to 4 days. The dry matter of samples taken at 1.5, 3 and 4 days was determined.

Green kiwifruit combined with acid

The pH of four 100-g samples of minced fish was changed to pH 4 using 0.7 ml sulphuric acid, 17 ml HCl, 5 ml citric acid or 2 ml formic acid. Ten grams of

GKF were then added to each sample and mixed in using a wooden spoon. These samples were kept in a 40 °C oven for up to 4 days and the dry matter of the samples taken at 1.5, 3 and 4 days was determined.

Whole kiwifruit or kiwifruit pulp

The pH of three 100-g samples of minced fish was changed to pH 4.0 using 0.7 ml sulphuric acid before adding 10 g of GKC, goldKF or goldKC. These samples were kept in a 40 °C oven for up to 4 days and the dry matter of the samples taken at 1.5, 3 and 4 days was determined.

Using kiwifruit to change pH

*The p*H of one 100-g sample of minced fish was changed to pH 4.0 using 100 g of goldKC but the second 100 g sample was mixed only with 10 g of GKC. After mixing the kiwifruit in using a wooden spoon, the samples were kept in a 40 °C oven for up to 4 days. The dry matter of the samples taken at 1.5, 3 and 4 days was determined.

4: RESULTS AND DISCUSSION

This chapter describes and discusses the trials carried out. Preliminary trials were done to study the effect of stirring, temperature and pH on the silage process. Once the optimum operating parameters had been determined, these conditions were used for the main trials, which studied the effect of types of acids on their own or mixed, and the effect of adding pulped kiwifruit as a source of exogenous proteolytic enzyme.

4.1. Preliminary trials

4.1.1. Effect of stirring conditions, ensiling time, temperature and pH

The literature recommends that silage is stirred many times daily for few days (Alvarez, 1972; Cervantes, 1979). The first trial compared the effect of exposing the silage to air during daily stirring with mixing the silage without being exposed to air. The former was done by opening the vessel (plastic bag) to accomplish the stirring. This also allowed the pH to be measured. The second sample was kept anaerobically and stirred by manually pulsing the closed bag. The pH could not be measured.

The trial was done for 10 days. Initial pH of both samples was very low (Table 4.1) due to the added acid. Although the same amount of sulphuric acid had been added to the samples, they had different initial pH values probably due to the difficulty of mixing the 500-g lots of minced fish in the plastic bag to obtain uniformity.

Table 4.1: Effect of exposure to air during daily stirring on the pH of minced fish samples after being stored for 10 days

Time (h)	Sample exposed to air	Sample not exposed to air
Initial (t= 0)	1.63	2.23
Final (t= 240)	4.24	4.18

The final pH of the sample that had not been exposed to air was slightly lower than the sample that was opened daily when being mixed (Table 4.1). The pH of the sample that was daily mixed increased with storage time in the first 100 hours and then tended to reach a stable value (Fig. 4.1). This indicates that the biochemical changes (such as dissolving the fish bones and solubilising the muscle protein) produced alkaline biochemical compounds, such as ammonia, that raise the pH.

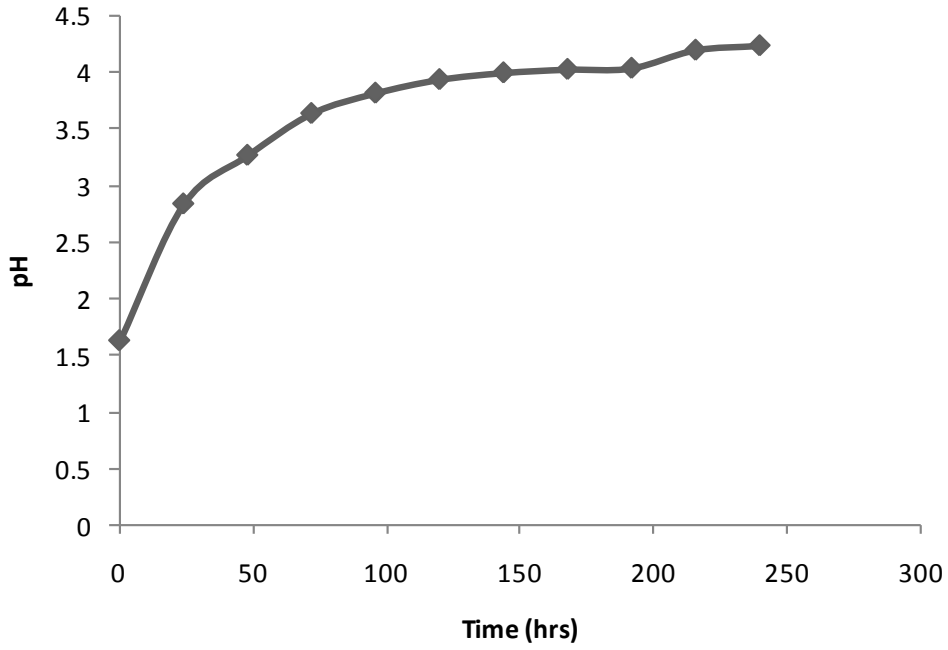

Figure 4.1: Increase in pH for sample exposed to air during daily mixing

Visual observations after ten days indicated that little liquefaction had occurred. This trial had been done at 'room temperature' (~20°C at the time of trial), the temperature used in various reported reviews (see section 2.4.3). However, it appears that silage production did not occur at the relatively low temperature used and probably would need a very long time (see section 2.4.2).

To investigate the effect of a higher temperature on liquefaction, both samples were stored in a 30°C oven for one day (11^{th} day of silage process). The pH of the anaerobic sample B was adjusted to 3.5, a pH commonly recommended (see section 2.4). Storing samples of different pH at 30°C would help identify whether the silage process depended on temperature and/or pH. For the purposes of this infestation, it was assumed that silage from the anaerobic and aerobically mixed

samples were similar (especially seeing there had been little liquefaction in either sample).

There was no significant change in visual appearance and the mean pH increase was only 0.3 after one day storage at 30^{o}C. It was therefore concluded that the temperature was still too low so it was raised to 40°C. Within a further day (day 12), both samples were liquefied. The pH of both samples increased over the further four days of ensiling. The pH of sample that had been lowered to pH 3.5 increased by about 0.1 units each day and was readjusted to 3.5 (Table 4.2).

Table 4.2: Effect of initial pH and time on silage dry matter

	Sample A (no pH adjustment)		**Sample B (pH adjusted to 3.5 every day)**	
Time(day)	**pH**	**Total dry matter (g/100 g)**	**pH**	**Total dry matter (g/100 g)**
0	4.44		3.5	
12th	4.72	25.8	3.67	27.4
13th	5.28		3.62	
14th	5.5		3.62	
15th	5.6	25.5	3.58	27.7

The total dry matter of the samples did not change substantially over the four days (Table 4.2), indicating that no volatiles had been lost. Good fish silages have low total insoluble dry matter and a high soluble dry matter content. Sample A had a lower dry matter content than sample B, indicating that the higher pH probably created volatiles, which reduced the amount of total solids. The similarity of the dry matter on days 12 and 15 for both samples shows that once maximum liquefaction is reached, dry matter remains constant as long as volatiles are not released.

The overall result from this trial showed temperature and pH affect fish liquefaction and that temperature should be raised to 40^{o}C and pH lowered to 3.5 to 4.

4.1.2. Effect of temperature

This trial was done at temperatures between 25°C and 45°C because it would take a long time to turn samples stored at temperatures lower than 25°C into silage. Proteolytic enzymes responsible for fish liquefaction will denature if samples are stored above 45°C.

Good fish silage would have low insoluble solids content. Data indicate samples kept between 30 and 40°C (Table 4.3) had lower total solids than those stored at either 25 or 45^{o}C (Fig. 4.2). The higher solids content of the 45^{o}C is probably due to the reduced proteolytic enzyme activity. The higher solids content of the sample stored at 25^{o}C is probably because the sample had not been stored long enough.

Table 4.3: Effect of storage temperature on dry matter content of minced fish stored for four days

Temperature	**Dry matter (g/100 g)**
25°C	21.1
30°C	20.9
35°C	21.0
40°C	20.5
45°C	23.3

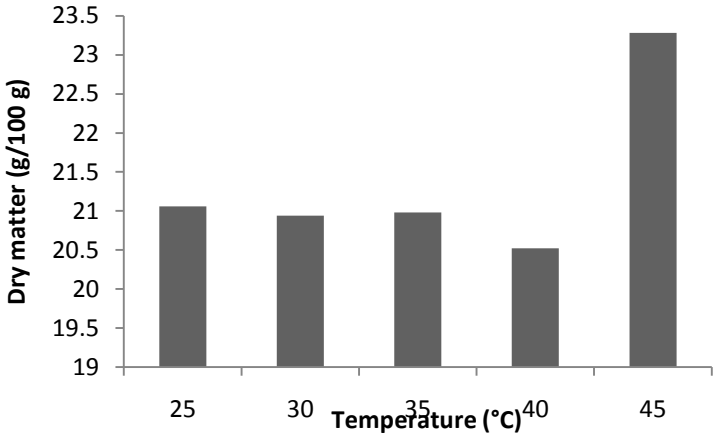

Figure 4.2: Dry matter content of samples at different temperatures

4.1.3. Effect of pH

The dry matter measured in the previous trials was done on a whole silage sample and represented total solids content. Total solids are made up of soluble solids and insoluble solids. For increased accuracy, further work was done on measuring both insoluble solids and soluble solids that had been separated via filtration or centrifugation. High dry matter content in the soluble solids would then be used as the criterion for identifying the best silage. Having a high soluble solids content is the goal for practical applications of fish silage such as fertilizers (there is more material for the plants to take up) or pet food (higher digestibility). The effect of pH on silage production was studied using both static filtration and centrifugation.

Static Filtration: Soluble solids and insoluble solids could be successfully separated by filtration but the analysis needed to be carried out overnight. If the sample is not properly covered, evaporative losses can occur. Samples were kept for two days because it was difficult to separate liquid from acidified thawed minced fish. Liquefied samples obtained after 24 h was not uniform. To obtain representative results, the sample was re-mixed, re-adjusted to the required pH and kept in the 40^{o}C oven a further 24 h before determining its dry matter.

A filtrate could be obtained from all samples (Table 4.4) except the control sample (no added acid and at pH 6.95), which could not be filtered at all. Filtrate

from samples with a pH between from 2 to 4 was clear and yellow whilst the filtrate from samples at pH 5 and 6 was dark brown.

The highest soluble dry matter content obtained was 7.8 g/100 g of original sample, which occurred when the pH was at 6. However, the insoluble solids content of this sample was also high, giving high total solids content. A further disadvantage of this higher pH is the rapid microbial spoilage. Most of the bacteria are neutrophiles, with an optimal growing pH between 5.5 and 8 whereas most fungi prefer a pH 4-6 (Prescott *et al*., 1999).

Table 4.4: Dry matter obtained from different pH samples after static filtration

pH	Weight (g/100 g)			Dry matter content (%)		Dry matter (g/100 g)		
	Liquid	Solid	Evaporative losses	Liquid	Solid	Soluble	Insoluble	Total solids
Control (6.95)	0.0	100	0.0	0.0	24.2	0.0	24.2	24.2
pH 2	16.6	52.8	30.6	11.8	32.9	2.0	17.4	19.4
pH 3	32.2	38.8	29.0	14.6	40.9	4.7	15.9	20.6
pH 3.5	38.8	31.4	29.8	16.0	48.8	6.2	15.3	21.5
pH 4	44.0	28.6	27.4	16.6	50.6	7.4	14.5	22.0
pH 5	21.2	50.4	28.4	16.8	31.7	3.6	16.0	19.6
pH 6	38.0	36.4	25.6	20.6	44.3	7.8	16.1	23.9

After excluding samples kept at the higher pH on grounds that they may not be microbially safe or stable, the values for soluble dry matter content indicated that processing at pH 3.5 or pH 4 would to be satisfactory because these samples had the higher soluble solids content (Fig. 4.3). These data support the literature (section 2.4.2), that indicates the appropriate pH for making fish silage is 3.5-4.0.

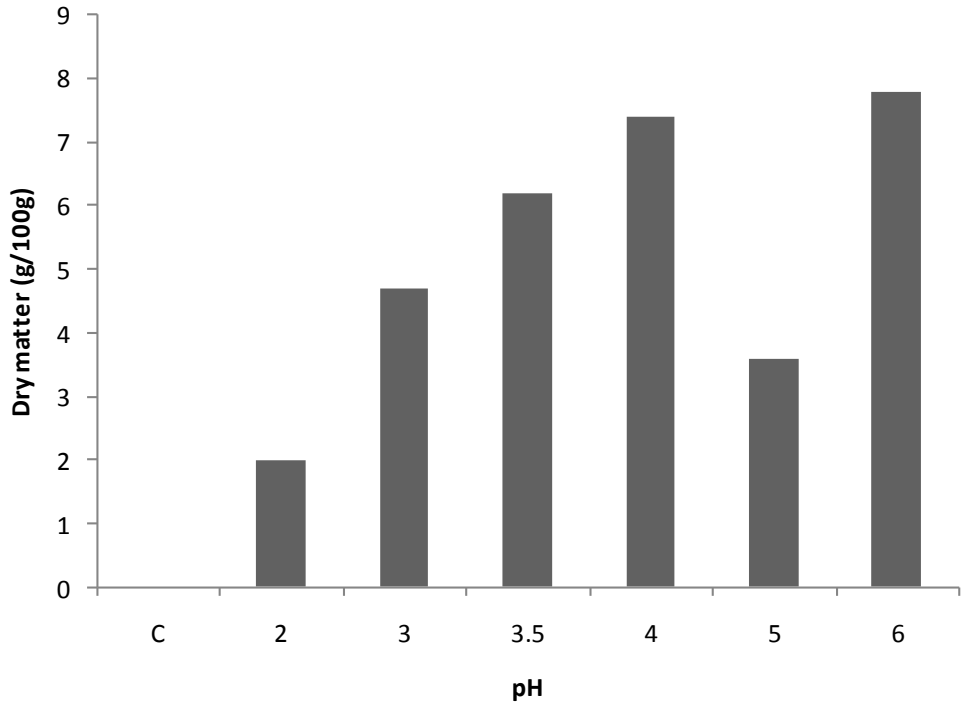

Figure 4.3: Effect of storage pH on the soluble dry matter (g/100 g) after keeping acidified minced fish at 40^{o}C for two days.

Centrifugation: When samples of minced fish that had been kept at 40^{o}C for two days were centrifuged, four layers were formed. The bottom solid phase was mainly made of fish scales and bones that had not dissolved. The next layer was a clear liquid phase. On top of this was an emulsion layer, which was covered with a thin layer of oil. In some samples, there were only traces of oil (Table 4.5).

Table 4.5: Visual observations of centrifuging minced fish samples stored at 40^{o}C for two days (sample size – 26 ml)

pH	Colour of liquid layer	Amount of emulsion (ml)	Oil
Control (pH 6.5)	Dark	5	Droplets
2	Yellow, clear	1.5	Thin layer
3	Yellow, clear	1.5	Traces
3.5	Yellow, clear	1	Thin layer
4	Yellow, clear	1.5	Traces
5	Dark brown, clear	2.5	Thin layer
6	Dark brown clear	4	Thin layer

As with filtration, the liquid phase in samples at pH 5 and 6 were dark but those at other pH values were clear yellow. In most samples, the thin oil layer was approximately 0.2 to 0.5 ml and traces of oil were present as droplets in the top layer. Because this oil layer could not be easily separated and because the emulsion was not included in the bottom (solid) phase, soluble dry matter calculations were done by considering the upper three layers (oil, emulsion and clear liquid) as the "liquid" fraction and the bottom layer as the "solid" fraction. After allowing for the weight/volume of the two major phases, the mass balance could be done on the sample (Table 4.6).

Table 4.6: Effect of pH on dry matter of phases obtained by centrifuging minced fish that had been stored at 40°C for two days

pH	Phase (g)			Dry matter %		Total dry matter (g/100 g)		
	Liquid	Solid	Losses	Liquid	Solid	Soluble	Insoluble	Total solids
Control (6.95)	66.0	13.0	21.0	20.8	53	17.5	8.5	26.0
pH 2	52.0	27.2	20.8	13.2	22.6	8.5	7.8	16.3
pH 3	57.6	20.6	21.8	16.6	24.7	12	6.3	18.3
pH 3.5	62.0	16.4	21.6	20.0	26.3	15.5	5.5	21.0
pH 4	64.2	15.2	20.6	20.0	27.1	16	5.3	21.3
pH 5	64.0	14.4	21.6	18.0	40.7	14.5	7.5	22.0
pH 6	65.0	14.4	20.6	22.8	52.7	18.5	9.5	28.0

The effect of pH on the amount of the liquid and solid phases obtained by centrifuging the samples was similar to that obtained by filtering the samples. Also, the content of soluble and insoluble solids in the phases was similar. This showed that both filtration and centrifugation could be used to separate the phases.

When the phases were separated by centrifugation, and excluding the sample at pH 5-6 because of the possibility of higher putrefaction, the highest soluble solids content (16 g/100 g of stored minced fish sample) was obtained in the pH 4 sample and the next highest soluble solids content (15.5g/100 g minced fish) occurred in samples stored at pH 3.5 (Fig. 4.4). These results showed that the pH of minced fish should be between 3.5 and 4 to obtain good quality fish silage.

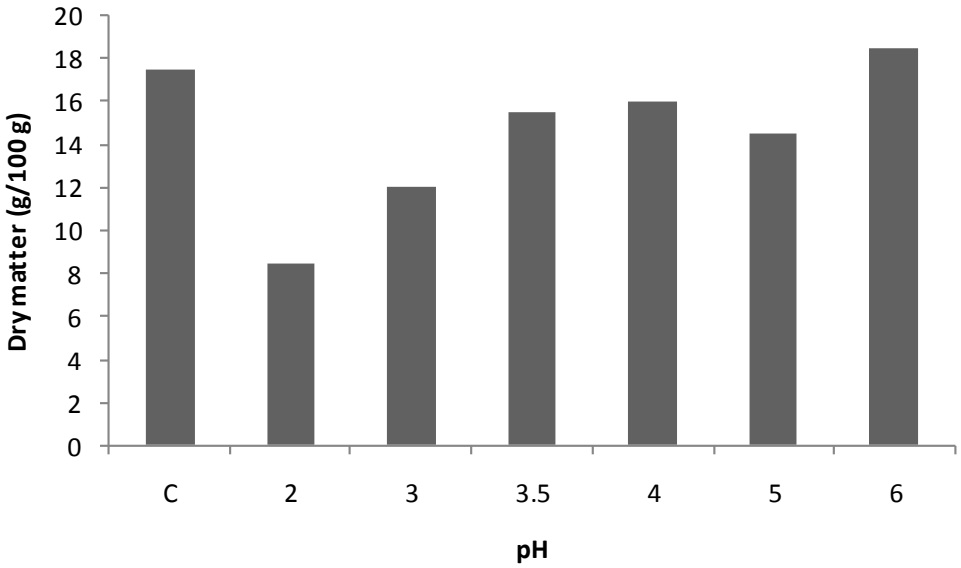

Figure 4.4: Effect of pH of minced fish stored at 40°C for two days on the amount of soluble dry matter (g/100 g) obtained by centrifuging the sample.

Although, it was much quicker and easier to centrifuge than filter samples, it had a major drawback. The solid phase tended to stick to the tube walls and could not be easily retrieved without considerable loss. Also, separating soluble solids from the insoluble material could not be achieved accurately when the emulsion phase was present. Therefore, filtration would be the preferable method for analysing the silage analysis. However, if the static filtration was only done for few hours, some of the soluble solids will remain in the residual solids on the filter paper. Extending the filtration time (for example, filtering for 20 h as was done in the analyses) until no further liquid was produced would be more simple. However, large evaporative losses occur. A better method, which would provide more accurate results, would be to adopt vacuum filtration.

4.2. Main trials

After the optimum processing conditions for producing fish silage had been determined, the following conditions were used to conduct the main trials: 100 g of thawed minced fish was stored in plastic bags at 40°C for four days. The effect of acids (single and in combination) and kiwifruit pulp on silage process was investigated.

4.2.1. Single acids

Mineral acids: Mineral acids are commonly used for producing fish silage because they are cheaper than organic acids. The effect of type of acid and processing time on the soluble and insoluble dry matter of the treated minced fish was determined for the control (no added acid) and each acid (Table 4.7).

The soluble dry matter of all samples increased with time and reached a maximum after four days (96 h). As in the preliminary results, less liquefaction occurred on the first day in all samples, regardless of the type of acid added, probably because uniform mixing had not been achieved.

Initial total dry matter of the minced fish was 29 g/100 g. This is converted to soluble matter during the silage process. The insoluble material in the control decreased with processing time, which is represented by an increase in soluble solids (Fig. 4.5). However, the total solids content of the control decreased, due to loss of volatile material. Biochemical changes occurring during liquefaction convert some of the solids in the bones, scales and flesh to ammonia, soluble peptides and other volatile substances that can evaporate. This represents loss of product. These compounds also produce a very unpleasant odour.

Total solids for fish acidified with sulphuric acid was constant during the process, indicating that no material had been lost even though about one-third (9.4 g/100 g) had been solubilised (Fig. 4.5). Hydrochloric acid induced more drastic changes. total solids treated decreased from 29 g/100 g minced fish to 16.5 g/100 g, which represents loss of product. The increase in soluble solids content was less than achieved with sulphuric acid. There was no significant liquefaction in the sample with added nitric acid. This sample had low soluble solids and a slightly reduced total solid content indicating some biochemical changes had occurred.

Table 4.7: The effect of mineral acid and storage time on soluble and insoluble dry matter in minced fish with an initial pH of 4, stored at 40^{o}C

Time (h)	Phase (g/20 g sample)		Dry matter %		Total dry matter (g/100 g)		
	Solid	Liquid	Solid	Liquid	Insoluble solids	Soluble solids	Total solids
Control							
0	20.0	0	29.0	0	29.0	0	29.0
36	16.2	3.2	32.7	13.3	26.5	2.0	28.5
72	13.6	6.0	33.0	13.3	22.5	4.0	26.5
96	10.0	10.0	32.0	13.0	16.0	6.5	22.5
Sulphuric acid							
0	20.0	0	29.0	0	29.0	0	29.0
36	10.3	9.5	39.8	10.0	20.5	5.0	25.5
72	8.0	11.9	48.8	13.3	19.5	8.0	27.5
96	8.4	11.3	46.4	16.7	19.5	9.4	28.9
Hydrochloric acid							
0	20.0	0	29.0	0	29.0	0	29.0
36	13.3	5.3	30.1	20.0	20.0	5.5	25.5
72	4.8	15.0	39.6	10.0	9.5	7.5	17.0
96	4.5	15.5	37.8	10.0	8.5	8.0	16.5
Nitric acid							
0	20.0	0	29.0	0	29.0	0	29.0
36	14.8	4.9	32.2	13.3	23.8	3.3	27.1
72	11.0	8.5	37.3	13.3	20.5	5.7	26.2
96	10.3	9.4	36.3	13.3	18.7	6.3	25.0

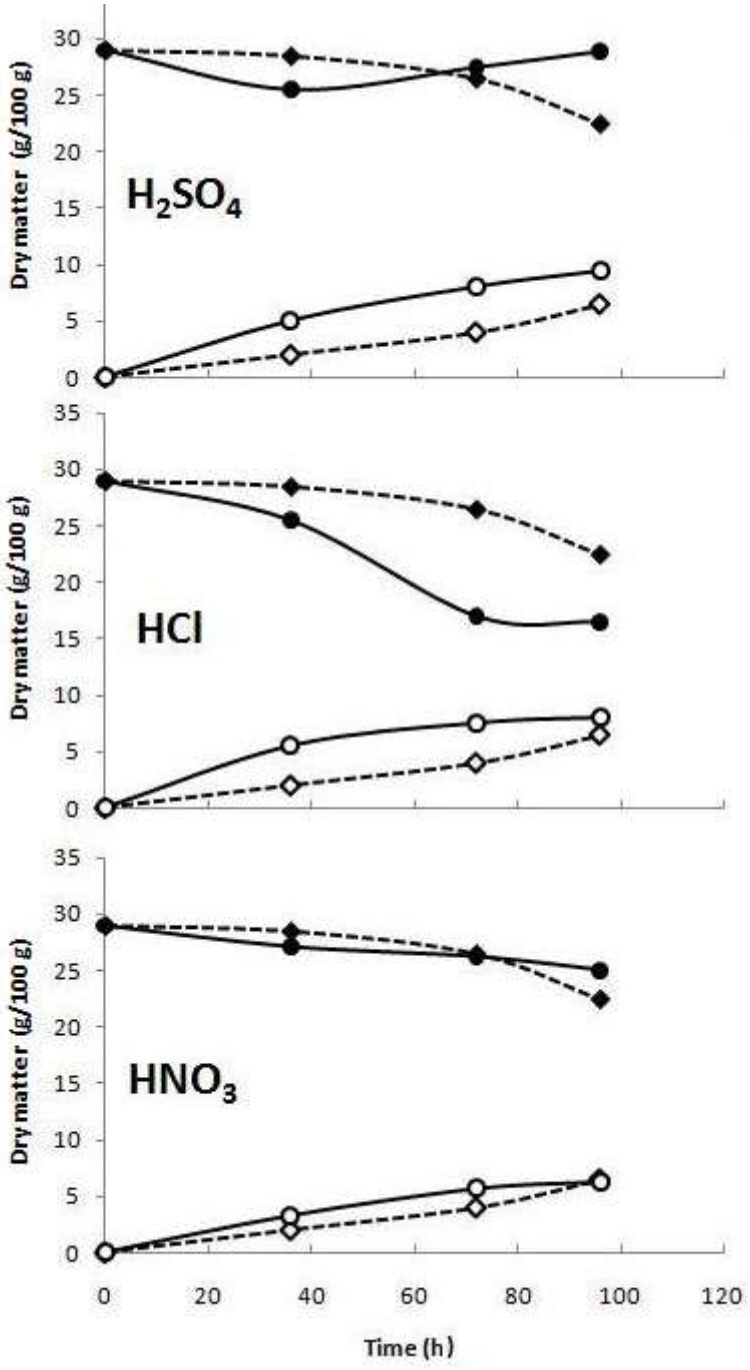

Figure 4.5: Effect of mineral acids and processing time on soluble solids content of the fish silage (soluble solids in Control; total solids in Control; soluble solids in sample; total solids)

The control sample had a very unpleasant odour because its neutral pH of 6.95 allowed putrefaction to occur. The odour of samples been acidified to pH 4 was acceptable.

Visual observations indicated that adding hydrochloric acid produced the most liquefied (least viscous) material. Samples acidified with sulphuric acid were slightly more viscous. Although both the control and fish acidified with nitric acid liquefied at 40°C, they were more viscous than the other silages. Even though visual observations indicate that adding hydrochloric acid produced a liquid silage, sulphuric acid gave a better quality silage because it has the highest amount of soluble solids and there was minimal loss of total solid.

***Organic acids**:* Organic acids are more expensive than mineral acids but were investigated because many reported trials have been done with these acids. Usually less organic acid is required to obtain the pH required to preserve the fish (see 2.4.2).

Soluble solids content increased with time and was maximum on the fourth day (Table 4.8). Samples with acetic acid lost only 7% of the initial solids content and produced the maximum amount of soluble solids (11 g/100 g fish). Samples with citric acid produced less soluble solids (9.5 g/100 g fish) but the total solids content was maintained. Formic acid, a very commonly used acid known to have a bacteriostatic action (see section 2.4.2), produced a similar amount of soluble solids to samples with citric acid and also lost some of the total solids due to biochemical reactions. The soluble solids content of samples with formic acid reached a maximum after 72 hours and then remained constant (Fig. 4.6).

Silages produced with organic acids had a similar odour to mineral acid silages and were acceptable. Formic acid silage tended to be darker than the other silages, which were all a light brown. White sandy particles (which could be from the bones) appeared in the solids when citric acid silage was filtered. The sample with added acetic acid had a strong acetic acid smell.

Of all the organic acids used, acetic acid gives the highest soluble solids with only slight loss in total solids and hence is the recommended additive. Overall, adding organic acids produced better silages than adding mineral acids

Table 4.8: The effect of organic acid and storage time on soluble and insoluble dry matter in minced fish with an initial pH of 4, stored at 40^{o}C

Time (h)	Phase (g/20 g sample)		Dry matter %		Total dry matter (g/100 g)		
	Solid	Liquid	Solid	Liquid	Insoluble solids	Soluble solids	Total solids
Acetic acid							
0	20.0	0	29.0	0	29.0	0	29.0
36	10.8	8.6	36.1	16.7	19.5	7.0	26.5
72	8.0	11.4	38.8	16.7	15.5	9.5	25.0
96	8.8	11.0	36.4	20.0	16.0	11.0	27.0
Citric acid							
0	20.0	0	29.0	0	29.0	0	29.0
36	14.1	5.5	35.5	13.3	25.0	3.5	28.5
72	7.5	12.4	49.3	13.3	18.5	8.0	26.5
96	8.5	11.1	44.7	16.7	19.0	9.5	28.5
Formic acid							
0	20.0	0	29.0	0	29.0	0	29.0
36	15.4	4.0	31.8	13.3	24.5	2.5	27.0
72	10.0	9.7	34.0	20.0	17.0	9.5	26.5
96	10.0	9.7	34.0	20.0	17.0	9.5	26.5

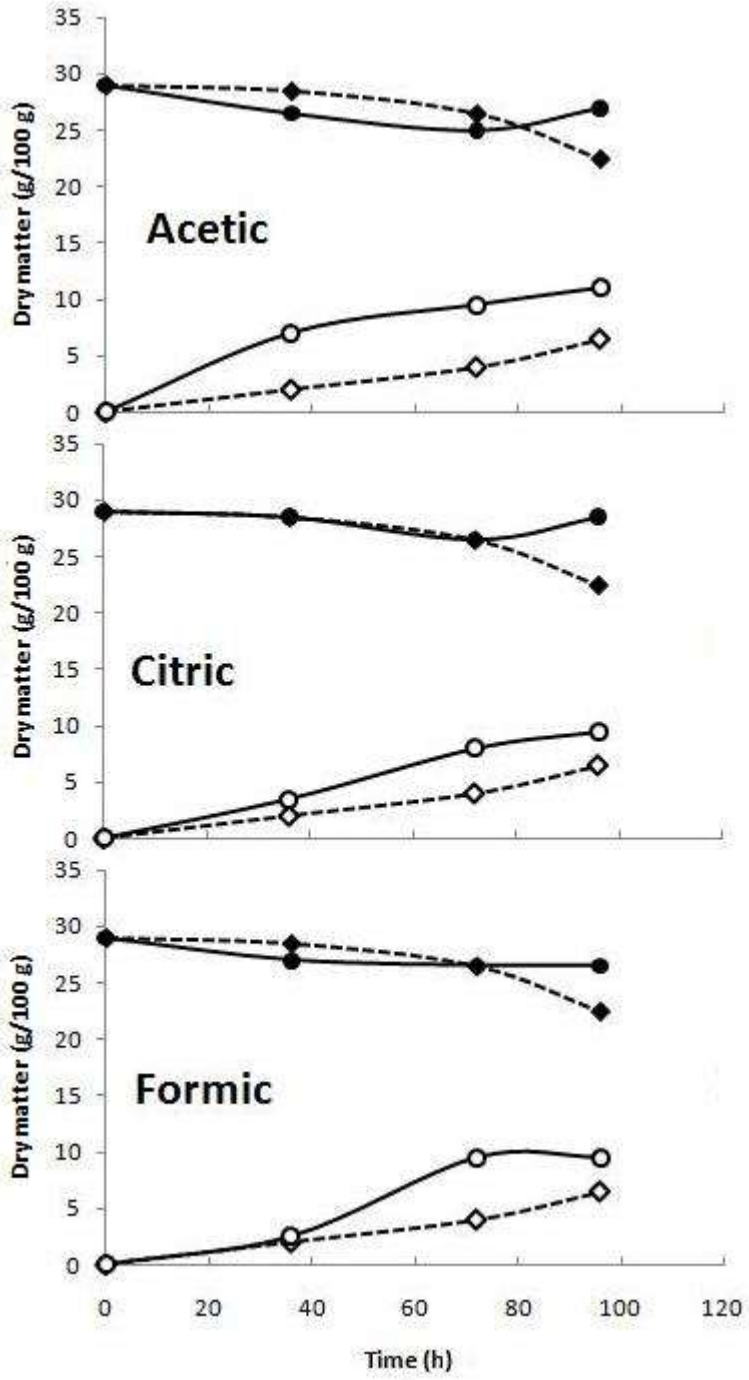

Figure 4.6: Effect of organic acids and processing time on soluble solids content of the fish silage (soluble solids in Control; total solids in Control; soluble solids in sample; total solids)

4.2.2. Acid combination trials

The literature indicates that combining organic and mineral acids produces good silage (see section 2.4.3). This may be due to chemical reactions between the acids. A set of mineral-organic acid combinations were investigated to identify how to produce good silage with the minimal acid.

H_2SO_4-organic acid combinations: Trials using combinations of sulphuric acid with organic acids showed that the sulphuric-acetic combination gave similar soluble solids content to using acetic acid used alone (10.5 g/100 g) with only slight loss of total solids (Table 4.9). The total solid content was maintained and a much higher soluble solid content was produced when using a combination of sulphuric and citric than citric acid or sulphuric acid used alone. A higher soluble solids content was also obtained when using sulphuric acid in combination with formic acid compared to the acids used alone. It can be seen from Figure 4.7 that the total solids in the sample with sulphuric acid combined with formic acid has 5 g less than the initial value (29 g/100 g) due to of biochemical changes. These are caused by formic acid as the total solids content in the trial using only formic acid also decreased a similar amount.

Silage made with sulphuric-formic acids was dark brown and those made with sulphuric-citric acids did not contain white sandy particles. Results for total solids and soluble solids for all sulphuric-organic acid combinations were similar. Also, the results were similar or better than using the acids alone. Of the combinations, the sulphuric-citric combination gave good soluble solids yields and maintained a total solids content.

HCl-organic acid combinations: There was a rapid increase in the soluble solids content of fish acidified with hydrochloric-acetic acid (Table 4.10; Fig. 4.8) to give 15 g soluble solids per 100 g with little loss in total solids. Adding HCl-acetic produced more soluble solids than using acetic acid alone and more than double the soluble solids when using only hydrochloric acid when .

Silage formed using HCl with formic acid was darker than the other silages. Also, the silage using HCl with citric acid (unlike sulphuric-citric acid combination)

contained white sandy particles. The acetic acid reacted with hydrochloric acid to produce the most of the mineral-organic acid combination used.

Table 4.9: Effect of H_2SO_4-organic acid combinations and storage on soluble and insoluble dry matter in minced fish acidified to pH 4 and stored at 40^oC

Time (h)	Phase (g/20 g sample)		Dry matter %		Total dry matter (g/100 g)		
	Solid	Liquid	Solid	Liquid	Insoluble solids	Soluble solids	Total solids
H_2SO_4 – Acetic							
0	20.0	0	29.0	0	29.0	0	29.0
36	9.2	10.2	40.2	10.0	18.5	5.1	23.6
72	6.9	13.0	43.5	10.0	15.0	6.5	21.5
96	7.5	12.3	42.7	16.7	16.0	10.5	26.5
H_2SO_4 –Citric							
0	20.0	0	29.0	0	29.0	0	29.0
36	10.0	9.4	39.0	13.3	19.5	6.3	25.8
72	7.2	12.3	43.1	13.3	15.5	8.2	23.7
96	6.9	13.1	49.3	16.7	17.0	11.0	28.0
H_2SO_4 – Formic							
0	20.0	0	29.0	0	29.0	0	29.0
36	9.6	9.6	40.6	10.0	19.5	5.0	24.5
72	7.7	11.7	40.3	13.3	15.5	8.0	23.5
96	6.8	12.7	42.6	16.7	14.5	10.6	25.1

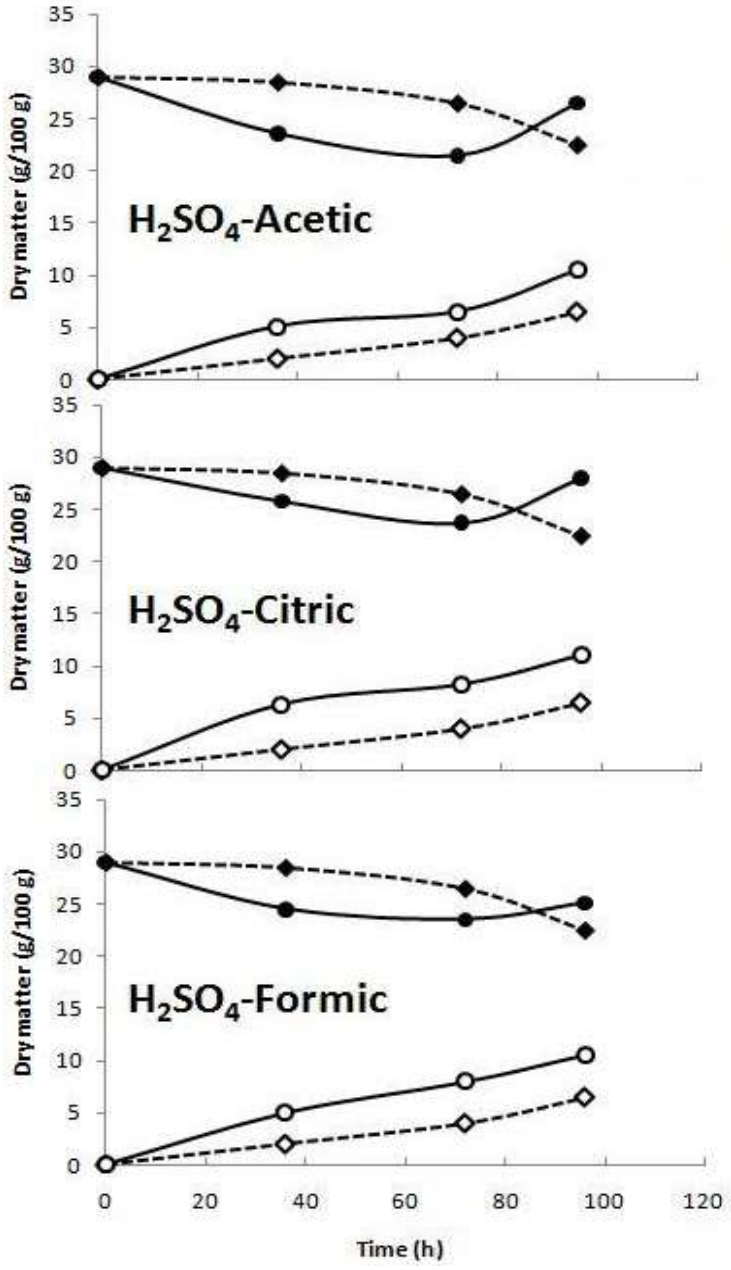

Figure 4.7: Effect of sulphuric-organic acid combinations and processing time on soluble solids content of the fish silage (soluble solids in Control; total solids in Control; soluble solids in sample; total solids)

Using HCl- citric acid produced much less soluble solids and there was a high loss of total solids (Table 4.10). The HCl-formic acid combination produced a similar amount of soluble solids as using the sulphuric-formic acid combination.

Table 4.10: Effect HCl-organic acid combinations and storage on soluble and insoluble dry matter in minced fish acidified to pH of 4 and stored at 40^{o}C

Time (h)	Phase (g/20 g sample)		Dry matter %		Total dry matter (g/100 g)		
	Solid	Liquid	Solid	Liquid	Insoluble solids	Soluble solids	Total solids
HCl- Acetic							
0	20.0	0	29.0	0	29.0	0	29.0
36	8.3	11.1	39.8	16.7	16.5	9.3	25.8
72	7.0	12.6	35.7	16.7	12.5	10.5	23.0
96	5.0	15.0	46.0	20.0	11.5	15.0	26.5
HCl – Citric							
0	20.0	0	29.0	0	29.0	0	29.0
36	9.0	10.2	41.1	13.3	18.5	6.8	25.3
72	6.5	13.5	46.2	13.3	15.0	9.0	24.0
96	6.4	13.3	46.8	13.3	15.0	9.0	24.0
HCl- Formic							
0	20.0	0	29.0	0	29.0	0	29.0
36	9.4	9.7	40.4	16.7	19.0	8.1	27.1
72	7.7	12.1	39.0	13.3	15.0	8.0	23.0
96	7.5	12.3	38.7	16.7	14.5	10.5	25.0

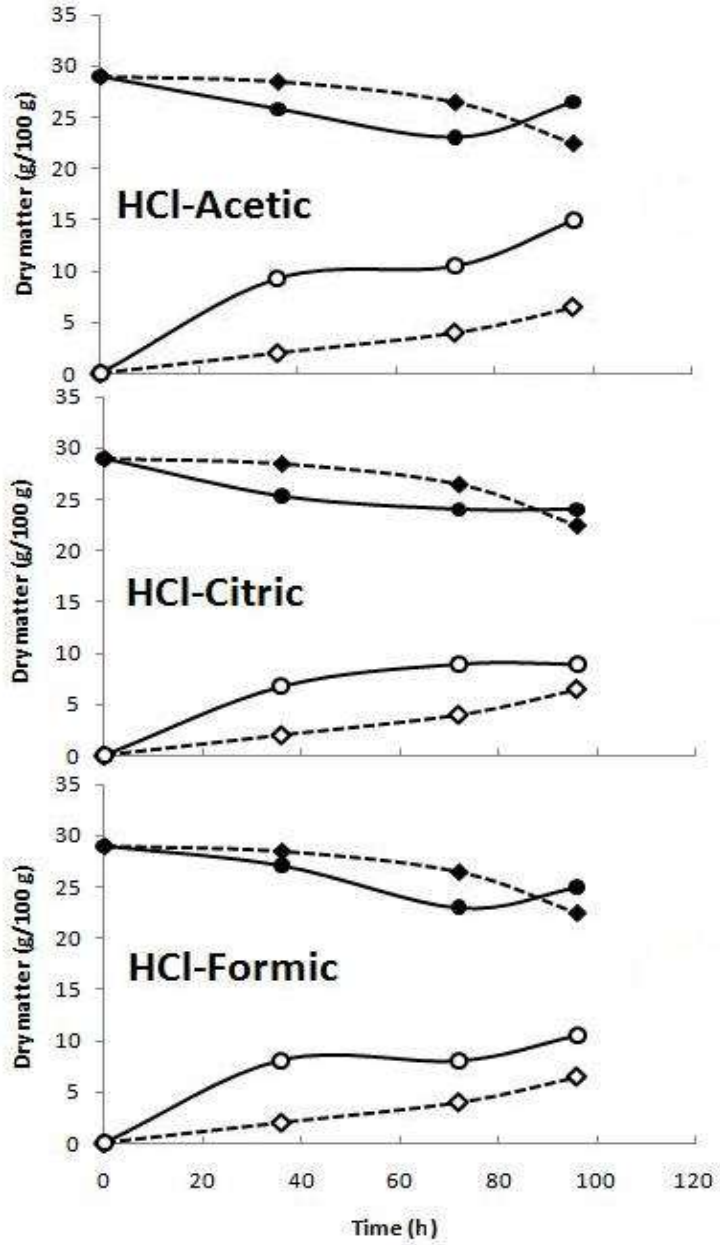

Figure 4.8: Effect of hydrochloric-organic acid combinations and processing time on soluble solids content of the fish silage (soluble solids in Control; total solids in Control; soluble solids in sample; total solids)

4.2.3. Adding kiwifruit and acid

Kiwifruit can be used as a source of exogenous protease because it contains the proteolytic enzyme actinidin. As large volumes of kiwifruit are available in the nearby regions, trials were done to see if it enhanced fish liquefaction. The first trial investigated whether adding kiwifruit pulp with a single acid produced better

silages. Literature (see section 2.4.3) indicated that the maximum actinidin content is in the kiwifruit flesh (that is, without seeds or skin).

- ***Mineral acid-green kiwifruit flesh combinations:*** There was only a slight increase in soluble solids when pulped kiwifruit flesh was added to minced fish that had been acidified with sulphuric acid. The maximum soluble solids was obtained after 72 hours and then remained constant (Table 4.11). The final soluble solids for the sulphuric-kiwfruit flesh after four days was lower than using acid alone. Also, there was a greater loss of total solids (Fig. 4.9). .

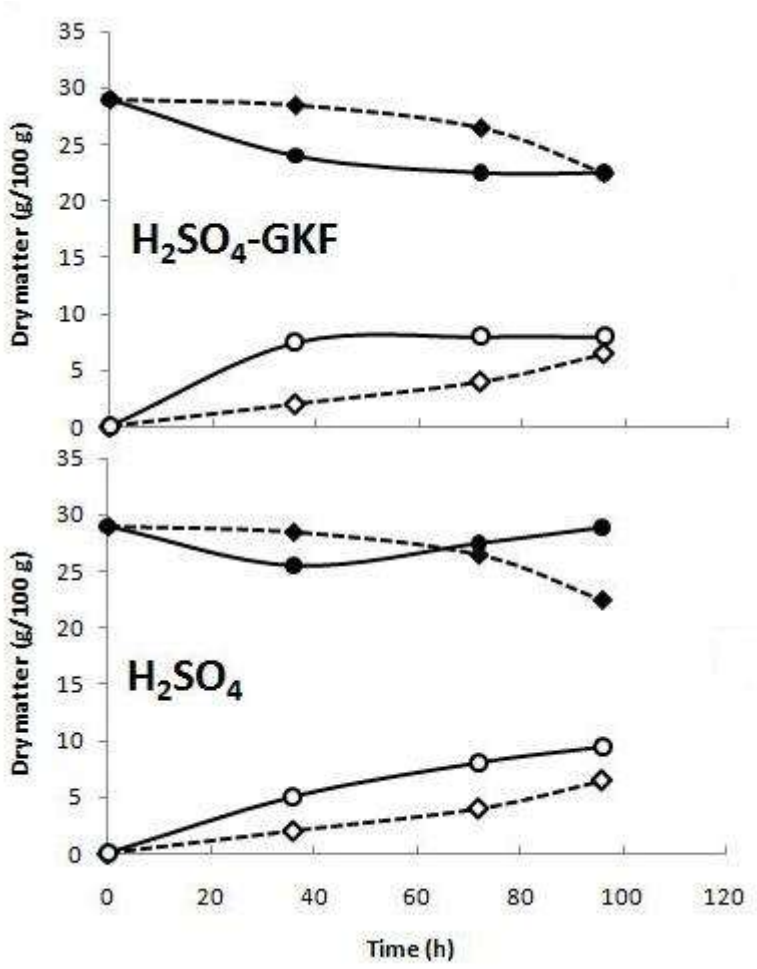

Figure 4.9: Effect of adding pulped kiwifruit flesh and processing time on soluble solids content of the fish silage acidified to pH 4 with sulphuric acid (soluble solids in Control; total solids in Control; soluble solids in sample; total solids)

Table 4.11: Effect of mineral acids with added pulped green kiwifruit flesh and storage on soluble and insoluble dry matter of minced fish acidified to pH 4 and stored at 40^{o}C

Time (h)	Phase (g/20 g sample)		Dry matter %		Total dry matter (g/100 g)		
	Solid	Liquid	Solid	Liquid	Insoluble solids	Soluble solids	Total solids
H_2SO_4 – GKF							
0	20.0	0	29.0	0	29.0	0	29.0
36	8.3	11.0	39.8	13.3	16.5	7.5	24.0
72	6.8	12.3	42.6	13.0	14.5	8.0	22.5
96	7.1	12.3	41.0	13.0	14.5	8.0	22.5
H_2SO_4							
0	20.0	0	29.0	0	29.0	0	29.0
36	10.3	9.5	39.8	10.0	20.5	5.0	25.5
72	8.0	11.9	48.8	13.3	19.5	8.0	27.5
96	8.4	11.3	46.4	16.7	19.5	9.4	28.9
HCl – GKF							
0	20.0	0	29.0	0	29.0	0	29.0
36	9.4	10.0	35.1	10.0	16.5	5.0	21.5
72	6.3	13.7	36.5	10.0	11.5	7.0	18.5
96	5.5	14.0	35.0	10.0	10.0	7.0	17.0
HCl							
0	20.0	0	29.0	0	29.0	0	29.0
36	13.3	5.3	30.1	20.0	20.0	5.5	25.5
72	4.8	15.0	39.6	10.0	9.5	7.5	17.0
96	4.5	15.5	37.8	10.0	8.5	8.0	16.5

The soluble solids when pulped kiwifruit flesh was added to minced fish that was acidified with hydrochloric acid reached a maximum after 72 hours (Table 4.11). The data indicates that adding pulped kiwifruit increased the liquefaction rate due

to the extra proteolysis. However, there was only minor differences between the reaction rates for silages without or with added kiwifruit flesh (Fig. 4.10).

The total solids of minced fish with added HCl or added HCl-GKF both decreased from 29 g/100 g to 17 g/100 g in four days, indicating that HCl was a major factor in the reaction (Fig. 4.10).

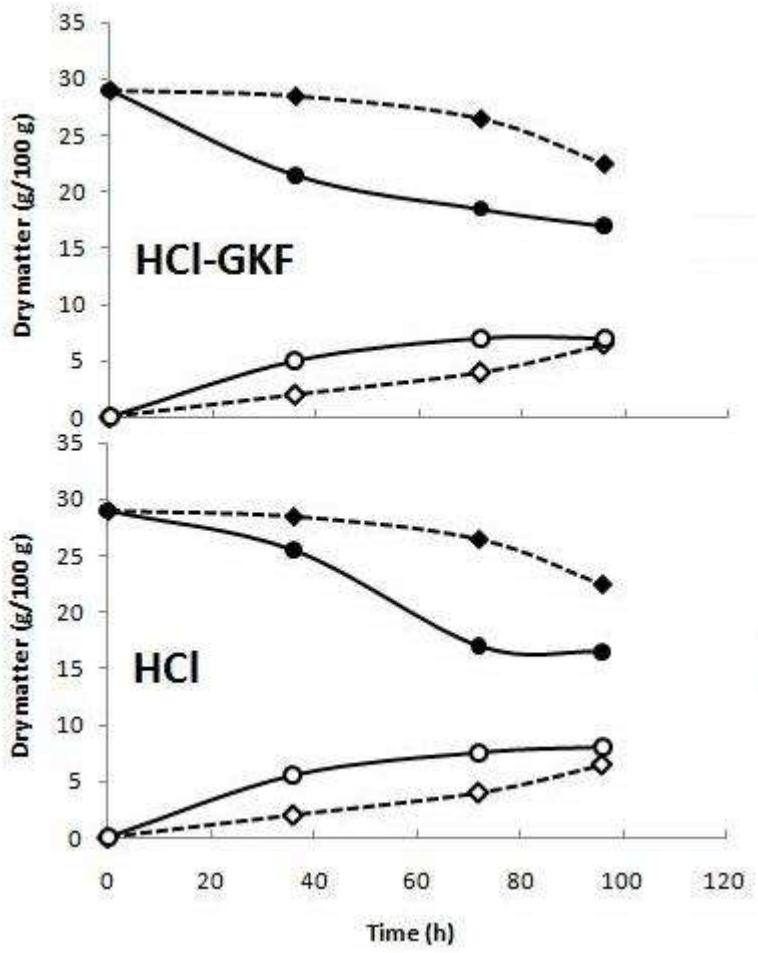

Figure 4.10: Effect of adding pulped kiwifruit flesh and processing time on soluble solids content of the fish silage acidified to pH 4 with hydrochloric acid (soluble solids in Control; total solids in Control; soluble solids in sample; total solids)

On the basis of the data for soluble solids content, adding pulped kiwifruit flesh and mineral acid did not provide any further advantages over using acids alone.

Organic acid-green kiwifruit flesh combinations: Adding pulped kiwifruit to minced fish acidified with citric acid produced slightly more soluble solids than using acid alone (Table 4.12). After 36 h, soluble solids in silage with added pulped kiwifruit was much higher (9 g/100 g) than using citric acid alone (3 g/100 g) (Fig. 4.11). Adding pulped kiwifruit flesh reduced total solids content slightly indicating, some of the proteolytic activity formed volatiles, which were then lost from the silage. The total solids decreased to a minimum of 26 g/100 g. Silages produced from adding citric acid and pulped kiwifruit flesh contained white sandy particles.

Table 4.12: Effect of using organic acids with added pulped green kiwifruit flesh on soluble and insoluble dry matter content of minced fish acidified to pH 4 and stored at 40^{o}C

Time (h)	Phase (g/20 g sample)		Dry matter %		Total dry matter (g/100 g)		
	Solid	Liquid	Solid	Liquid	Insoluble solids	Soluble solids	Total solids
Citric – GKF							
0	20.0	0	29.0	0	29.0	0	29.0
36	8.9	10.7	40.4	16.7	18.0	9.0	27.0
72	8.0	11.4	42.5	17.0	17.0	9.5	26.5
96	7.7	12.0	44.0	17.0	17.0	10.0	27.0
Citric acid							
0	20.0	0	29.0	0	29.0	0	29.0
36	14.1	5.5	35.5	13.3	25.0	3.5	28.5
72	7.5	12.4	49.3	13.3	18.5	8.0	26.5
96	8.5	11.1	44.7	16.7	19.0	9.5	28.5
Formic – GKF							
0	20	0	29	0	29	0	29
36	12.6	7	30.2	16.7	19	6	25
72	10.4	9.2	30.8	17	16	8	24
96	8.3	11	39	17	16	9.5	25.5
Formic							
0	20	0	29	0	29	0	29
36	15.4	4	31.8	13.3	24.5	2.5	27
72	10	9.7	34	20	17	9.5	26.5
96	10	9.7	34	20	17	9.5	26.5

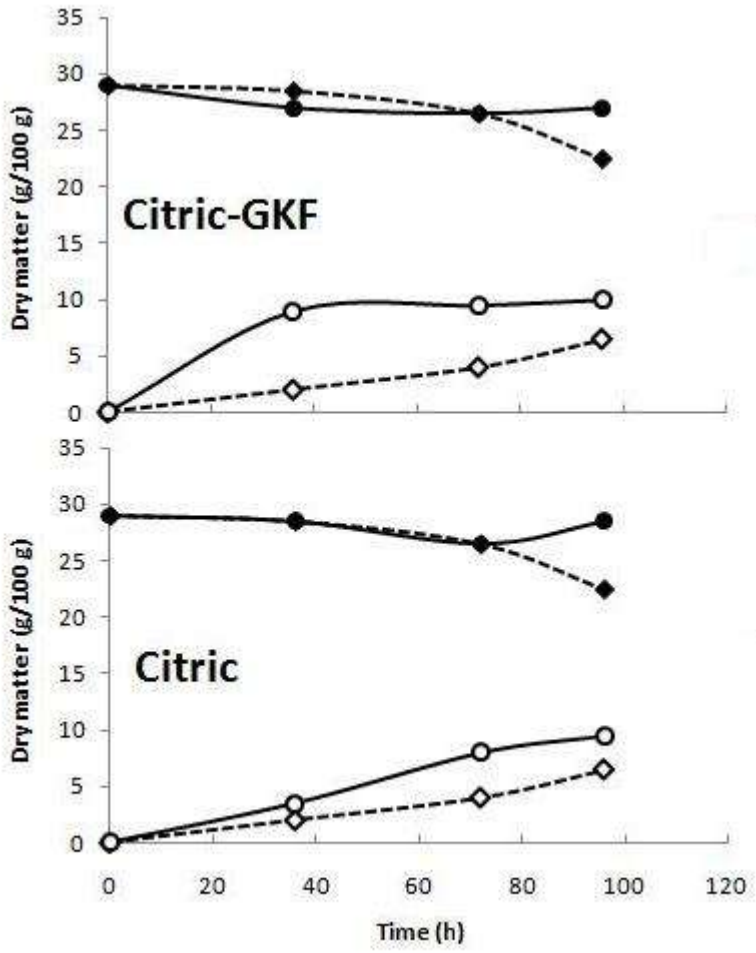

Figure 4.11: Effect of adding pulped kiwifruit flesh and processing time on soluble solids content of the fish silage acidified to pH 4 with citric acid (soluble solids in Control; total solids in Control; soluble solids in sample; total solids)

After 36 h, the soluble solids content produced by adding pulped kiwifruit to minced fish acidified with formic acid was higher (6 g/100 g) than using formic acid alone (2.5 g/100 g) (Fig. 4.12). The increase in soluble solids within the first 36 hours in all trials might have occurred because of the proteolytic activity of the kiwifruit which may have been inhibited or denatured after that. Daily additions of kiwifruit pulp may produce considerable changes in final soluble solid content compared to adding only organic acid. Total solids decreased slightly when kiwifruit pulp was added regardless of the organic acid used.

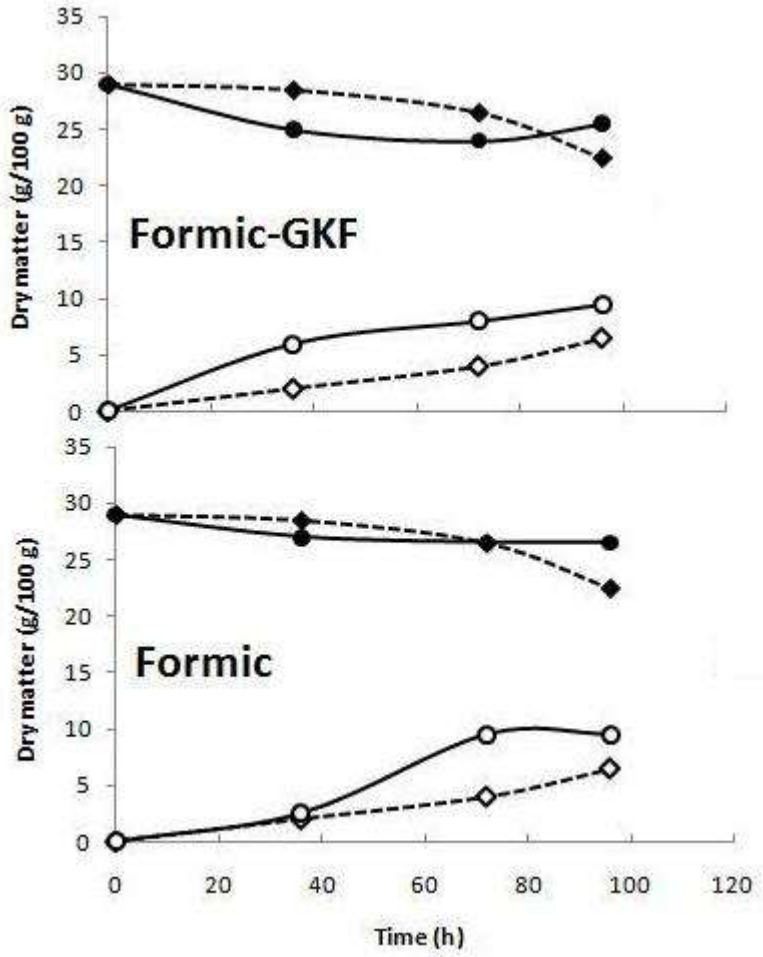

Figure 4.12: Effect of adding pulped kiwifruit flesh and processing time on soluble solids content of the fish silage acidified to pH 4 with formic acid (soluble solids in Control; total solids in Control; soluble solids in sample; total solids)

Of all the mineral or organic acids used, adding GKF gave the best results when used with citric acid.

Green kiwifruit and golden kiwifruit (crude and flesh)-acid combination: Another major kiwifruit variety is golden kiwifruit. Trials were done to test the effect of this variety on the silage process. For practical applications, whole pulped green kiwifruit (skin, flesh and seeds) and golden kiwifruit were used to investigate whether the skin and seeds have any inhibitory effects on the process. Silage made with sulphuric acid was used for comparison (Table 4.13).

Table 4.13: Effect of using pulped whole green or golden kiwifruit on soluble and insoluble dry matter content of minced fish acidified to pH 4 with sulphuric acid and stored at 40^{o}C

Time (h)	Phase (g/20 g sample)		Dry matter %		Total dry matter (g/100 g)		
	Solid	Liquid	Solid	Liquid	Insoluble solids	Soluble solids	Total solids
H_2SO_4 – GKF (green kiwifruit flesh)							
0	20	0	29	0	29	0	29
36	8.3	11	39.8	13.3	16.5	7.5	24
72	6.8	12.3	42.6	13	14.5	8	22.5
96	7.1	12.3	41	13	14.5	8	22.5
H_2SO_4-GKC (whole green kiwifruit)							
0	20	0	29	0	29	0	29
36	7.8	11.5	41	13.3	16	7.5	23.5
72	6.8	13	44.1	7	15	5	20
96	8.2	11.3	37	17	15	10	25
H_2SO_4-GoldKF (golden kiwifruit flesh)							
0	20	0	29	0	29	0	29
36	9.5	10.5	40	13.3	19	7	26
72	7.8	11.6	42.3	13	16.5	7.5	24
96	7.7	11.3	42	13	16	7.5	23.5
H_2SO_4-GoldKC (whole golden kiwifruit)							
0	20	0	29	0	29	0	29
36	9.5	9.8	42.1	13.3	20	6.5	26.5
72	7	13	47	13	16.5	8.5	25
96	8.3	11.6	39	13	16	7.5	23.5

Again, the major effect of adding kiwifruit pulp occurred within the first 36 h. By day four, pulped whole green kiwifruit gave higher soluble solids than pulped green kiwifruit flesh (Table 4.13). The soluble solids produced using pulped whole green kiwifruit was higher than from pulped whole golden kiwifruit.

Pulped whole golden kiwifruit gave exactly same results as given by pulped golden kiwifruit flesh (Fig. 4.13). The results indicate that pulped whole green kiwifruit could be added daily to silage to obtain a good fish silage.

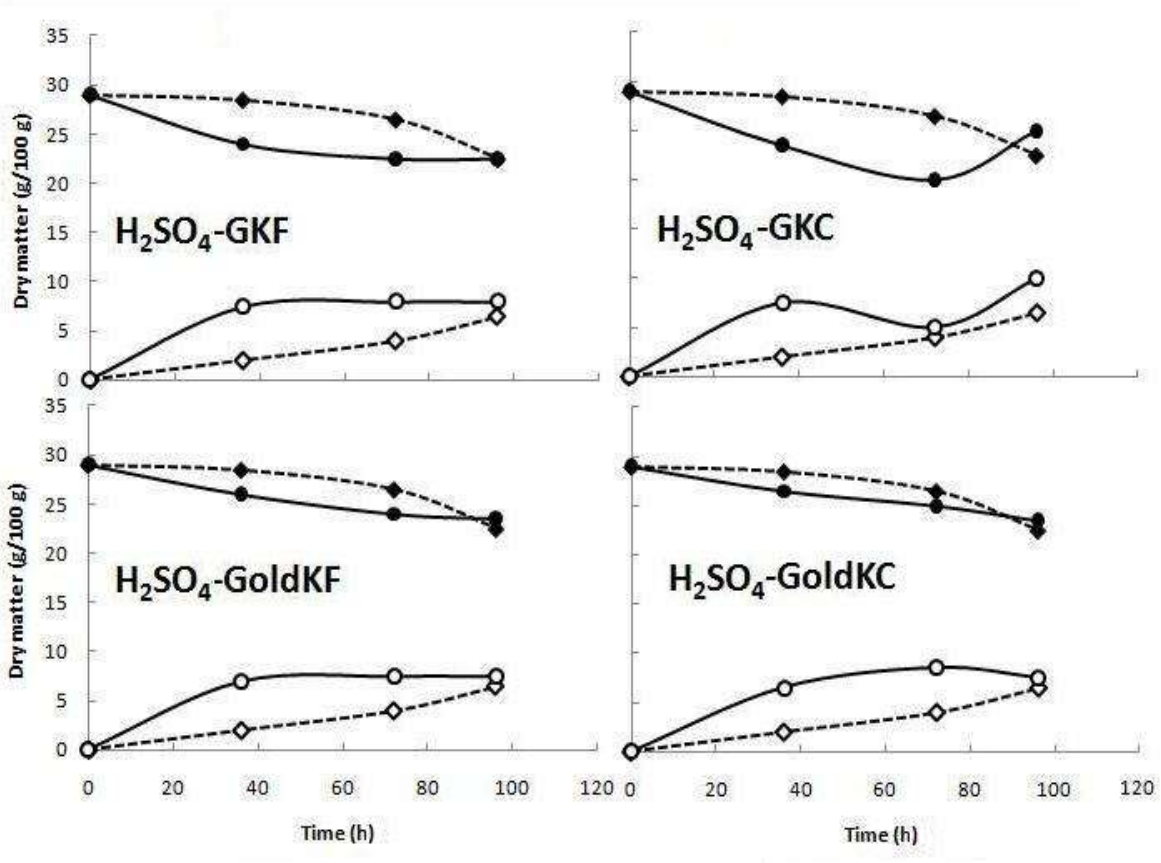

Figure 4.13: Effect of different kiwifruit samples and processing time on soluble solids content of the fish silage acidified to pH 4 with sulphuric acid (soluble solids in Control; total solids in Control; soluble solids in sample; total solids)

Kiwifruit only:

In all the main trials, different acids were added to produce a pH of 4.0. This trial was carried by using only pulped whole golden kiwifruit, which was added to give pH 4. This required 50% addition of kiwifruit. The soluble and total solids measured would therefore come from both the fish and the kiwifruit. Therefore,

data for this trial could not be directly compared with any of the other trials (Table 4.14).

Only minor liquefaction occurred. The product had a fruity-fishy smell and was a lighter colour because of the added kiwifruit.

Table 4.14: Effect of using only pulped whole golden kiwifruit on soluble and insoluble dry matter content of minced fish. Silage had a pH 4 and was stored at 40°C

Whole golden kiwifruit							
Time (h)	Phase (g/20 g sample)		Dry matter %		Total dry matter (g/100 g)		
	Solid	Liquid	Solid	Liquid	Insoluble solids	Soluble solids	Total solids
0	20	0	29	0	29	0	29
36	7.2	12.3	36	10	13	6	19
72	5.9	14.1	33.9	10	10	7	17
96	5.7	14	35.1	17	10	12	22

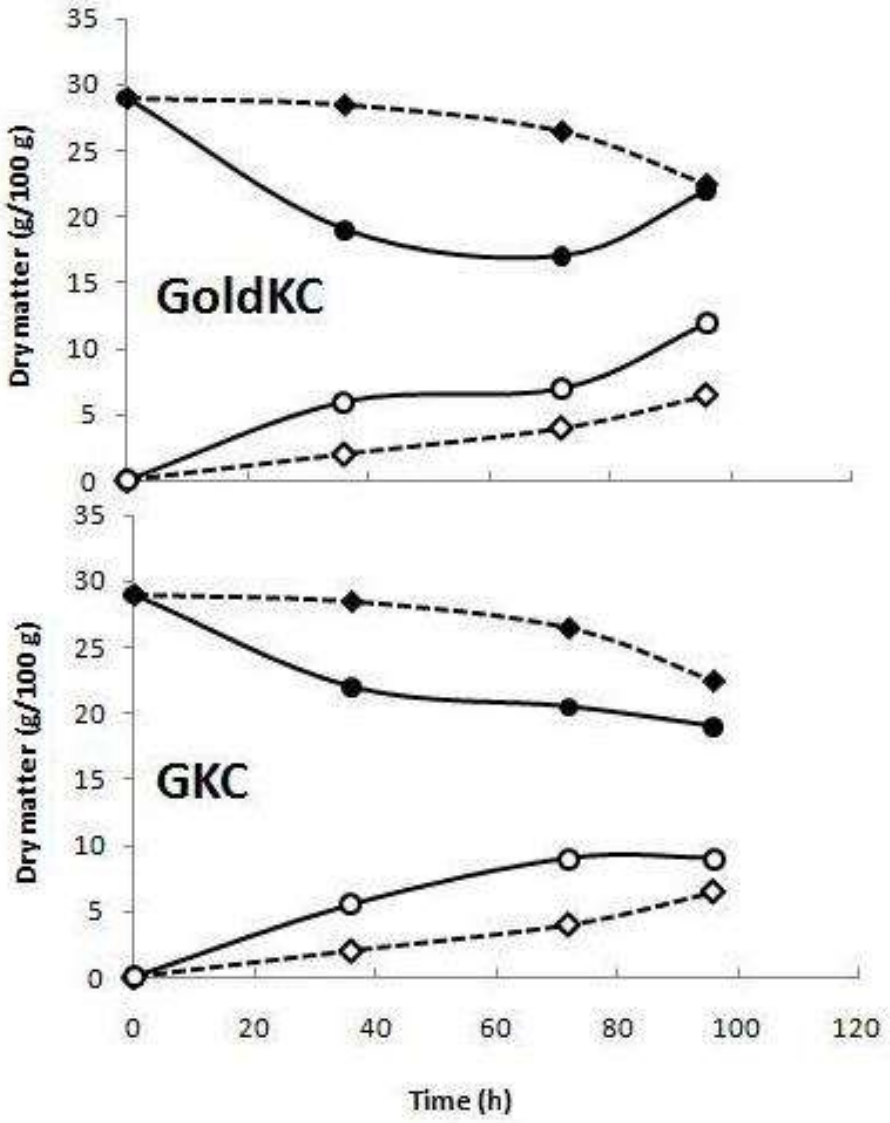

Figure 4.14: Effect adding pulped kiwifruit on soluble and insoluble dry matter in minced fish. Silage was stored at 40°C (soluble solids in Control; total solids in Control; soluble solids in sample; total solids)

As it is not practically feasible to add equal amounts of kiwifruit to fish for silage process, only 10 g of whole green kiwifruit was added to study its effect.

Table 4.15: Effect of using only pulped whole green kiwifruit on soluble and insoluble dry matter content of minced fish. Silage was not at pH 4 and was stored at 40^{o}C

Whole green kiwifruit							
Time (h)	Phase (g/20 g sample)		Dry matter %		Total dry matter (g/100 g)		
	Solid	Liquid	Solid	Liquid	Insoluble solids	Soluble solids	Total solids
0	20	0	29	0	29	0	29
36	11	8.6	30	13.3	16.5	5.5	22
72	5.1	14	45.1	13.3	11.5	9	20.5
96	5.5	13.7	36.4	13	10	9	19

The product liquefied and gave higher soluble solids yield than the control sample (Fig. 4.14; Table 4.15). This shows that the enzymes present in kiwifruit contributed to the liquefaction process. But this silage is not acceptable without acids, as it is acids which preserve the silage from putrefaction. The whole green kiwifruit sample was similar to the control in colour (dark) and smell.

4.3 Summary

The results of the various trials show that proper liquefaction of minced fish to produce silage occurs when the mixture is at pH 4 and stored at 40°C for three to four days. There should be adequate stirring to ensure homogeneity and remove localised pH variations. The pH should be re- adjusted to 4 to help increase the biochemical reactions occurring.

The best results when using single acids to acidify the silage mixture are obtained with sulphuric acid (mineral) or acetic acid (organic). These acids produce higher soluble solid content and there is little loss of total solid (which represents product loss). Acetic acid produced higher soluble solids than sulphuric acid. Biochemical reactions occurred when hydrochloric acid was added formed volatiles compounds and dramatically decreased total solids content. The

thoroughly liquefied silage can be compared to silage not properly liquefied from Figure 4.15 and Figure 4.16.

Figure 4.15: Properly liquefied silage

Figure 4.16: Silage before proper liquefaction (control sample kept at 40°C)

The results from most of the mineral and organic acid combinations were either similar or better than using the corresponding single acids. A combination of hydrochloric acid with acetic acid produced the highest soluble solids with only a 8% loss of the original total solids.

Adding pulped kiwifruit increased the reaction rate and all samples had a higher soluble solids content after 36 h than using single acids or combinations of acid. However, the enzymic activity of the kiwifruit then decreased, probably due to inhibitory effects from other components or the pH. The acid that responded best with kiwifruit was citric acid. Whole pulped green kiwifruit was better than kiwifruit flesh or golden kiwifruit for silage production. It is proposed that better results occur if aliquots of whole green kiwifruit pulp was added daily, along with an appropriate acid.

Results from all the trials showed that best results was produced using hydrochloric-acetic acid combination and produced the highest soluble solids without loss of total solids.

5: CONCLUSIONS AND RECOMMENDATIONS

Koi carp is a pest invading the New Zealand waters and causing deleterious effects to the aquatic ecosystem. A literature search was done to find possible applications for using the koi carp being caught as part of its eradication step. Of the uses described, the aim was to select an application that would use whole koi carp in a cost effective way with the constraint that it should not require a long term supply of fish. Silage production was chosen as one of feasible uses because it is a simple process that does not need a complex infrastructure. The product can be used in the biofertilizer and petfood industries.

Liquefaction of fish during silage process is achieved by endogenous enzymes and helped by added acid. The laboratory trials studied the effect of different processing conditions such as stirring conditions, temperature and pH, effect of acids (single and in combination) and effect of kiwifruit as a source of proteolytic enzyme in silage process.

For the fish to liquefy properly without putrefaction, minced fish should be mixed with the appropriate amount of acid to give a pH between 3.5 and 4.0 and then kept for three to four days at 40°C. The mixture should be thoroughly mixed so the whole fish contacts the acid. Stirring daily assists mixing. As pH increases due to biochemical reactions, it is necessary to readjust the pH by adding acid.

The production of soluble solids without excess loss of total solids was used to indicate a good silage. . Of the f three mineral acids investigated, sulphuric acid gave the best result. There was a large loss of total solids when hydrochloric acid was used. Of the three organic acids used, the best silage was formed using acetic acid. However, this was better than the silage produced by adding sulphuric acid.
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Most combinations of mineral and organic acid investigated produced similar or better silages than using the acid alone. Of all the combinations tested, combining hydrochloric acid with acetic acid produced silage with the highest soluble solids content (15 g/100 g of minced fish) without drastic loss of total solids.

Adding pulped kiwifruit as a source of proteolytic enzyme to silage, along with a mineral or organic acid only improved the process in the first 36 h. After this, proteolytic activity responsible for hydrolysis ceased, probably because the enzyme had been inhibited or denatured. Only the combination of citric acid with kiwifruit pulp gave better results (in terms of increased soluble solids content) after four days of ensiling than using acids without kiwifruit pulp. This increase was only slight.

The studies showed using pulped whole green kiwifruit gave better results than pulped whole golden kiwifruit or flesh without any seeds or skins from green or golden kiwifruit. The results indicate that daily addition of pulped whole kiwifruit to the minced fish, along with the desired acid, may give a suitable product under commercial production conditions.

The studies indicate that a good silage could be made by adding 50:50 v/v hydrochloric acid-acetic acid until the pH of minced fish was pH 3.5-4.0 and keeping the mixture at 40°C for four days. The pH should be maintained throughout the process.

It is recommended that further work should be done on using different acid combinations as well as using different proportions of acids. The effect of using different acid strengths could also be investigated. Finding better acid could help reduce the costs involved in silage manufacture. It is also recommended that the biochemical changes occurring throughout the process, including the volatile substances produced, why total solids content reduced in some silages, and what are the alkaline products being produced be studied in detail. As liquefaction is due to the action of proteolytic enzymes, it is recommended that other exogenous enzyme sources are investigated. Lastly, it is recommended that the processing costs and economic viability of a silage plant based on processing the captured koi carp is be done.

6: REFERENCES

Agro Products. (2008). Dry fish, 2010, from http://www.agriculturalproducts india.com/marine-food-supplies/marine-food-supplies-dry-fish.html

Ali, M., Iqbal, F., Salam, A., Iram, S. & Athar, M. (2005). Comparative study of body composition of different fish species from brackish water pond. *International Journal of Environmental Science and Technology, 2* (3), 229-232.

Alvarez, R. J. (1972). A comparative study of the nutritional value of the protein from ensiled fish. *Cuban Journal of Agricultural Science, 6*, 219-222.

Aminlari, M., Shekarforoush, S.S., Gheisari, H.R. & Golestan, L. (2009). Effect of actinidin on the protein solubility, water holding capacity, texture, electrophoretic pattern of beef, and on the quality attributes of a sausage product. *Journal of Food Science, 74*(3), C221-226.

Baker, B. (1996). Plant Nutrition from the Sea: Marine products can be used to supplement soil nutrients. Retrieved from http://www.noamkelp.com/ nutrition.html

Bala, B.K. & Mondol, M.R.A. (2001). Experimental investigation on solar dying of fish using solar tunnel dryer. *Drying Technology, 19*(2), 427-436.

Balios, J. (2003). *Nutritional value of fish by-products and their utilization as fish silage in the nutrition of poultry.* 8th International Conference on Environmental Science and Technology, Lemnos Island, Greece.

Bekhit, A.D., Morton, J.D. & Dawson, C.O. (2008). Effect of processing conditions on trace elements in fish roe from six commercial new zealand fish species. *Journal of Agricultural and Food Chemistry, 56*(12), 4846-4853.

Bello, R. & Brito, L. (1994). Production of biological silage from fish scraps. *Archivos Latinoamericanos de Nutrición, 44*(4), 264-269B.

Bello, R.A. & Fernández, Y. (1995). Evaluation of biological fish silage in broiler chicken. *Archivos Latinoamericanos de Nutrición, 45*(2), 134-139.

Bervoets, L., Van Campenhout, K., Reynders, H., Knapen, D., Covaci, A. & Blust, R. (2009). Bioaccumulation of micropollutants and biomarker responses in caged carp (*Cyprinus carpio*). *Ecotoxicology and Environmental Safety, 72*(3), 720-728.

Billard, R. (Ed.). (1995). *Carp biology and culture* (pp. 1-7). Paris: INRA

Bigbee, J.W., Sharma, K.V., Gupta, J.J. & Dupree, J.L. (1999). Morphogenic role for acetylcholinesterase in axonal outgrowth during neural development. *Environmental Health Perspectives, 107*(Suppl 1), 81-87.

Blatt, C.R. (1991). Comparison of several organic amendments with a chemical fertilizer for vegetable production. *Scientia Horticulturae, 47*(3-4), 177-191.

Boran, G. & Regenstein, J.M. (2009). Optimization of gelatin extraction from silver carp skin. *Journal of Food Science, 74*(8), E432-441.

Borghesi, R., Portz, L., Oetterer, M. & Cyrino, J.E.P. (2008). Apparent digestibility coefficient of protein and amino acids of acid, biological and enzymatic silage for Nile tilapia (Oreochromis niloticus). *Aquaculture Nutrition, 14*(3), 242-248.

Bourre, J.M. (2005). Effect of increasing the omega-3 fatty acid in the diets of animals on the animal products consumed by humans. *Médecine Sciences : M/S, 21*(8-9), 773-779.

Brown, R.S., Power, G. & Beltaos, S. (2001). Winter movements and habitat use of riverine brown trout, white sucker and common carp in relation to flooding and ice break-up. *Journal of Fish Biology, 59*, 1126-1141.

Cervantes, A. (1979). Ensilaje y harina de pescado en la ceba de cerdos. (pp. 75). La Habana.

Chadderton, W.L., Brown, D.J. & Stephens, R.T. (2004). *Identifying freshwater ecosystems of national importance for biodiversity: Criteria, methods, and candidate list of nationally important rivers*. Wellington, New Zealand: Department of Conservation.

Champion, P., Clayton, J. & Rowe, D. (2002). *Lake Manager's Handbook: Alien Invaders*. Wellington, New Zealand: Ministry for the Environment.

Chumchal, M.M., Nowlin, W.H. & Drenner, R.W. (2005). Biomass-dependent effects of common carp on water quality in shallow ponds. *Hydrobiologia, 545*, 271-277.

Chun-Mei, L., Zhao-Hui, Z., Qiong-Hong, W., Zhao, H., Hai-Feng, G. & Shan-Bai, X. (2008). Preparation and thermal stability of collagen from scales of grass carp (*Ctenopharyngodon idellus*). *European Food Research & Technology, 227*(5), 1467-1473.

Crexi, V.T., Grunennvaldt, F.L., Soares, L.A.S. & Pinto, L.A.A. (2007). Refinement of corvine (*Micropogonias furnieri*) oil manufactured by acid ensilage and thermal processing of meal. *Revista do Instituto Adolfo Lutz, 66*(1), 50-56.

Crexi, V.T., Monte, M. L., Soares, L. A. d. S. & Pinto, L. A. A. (2010). Production and refinement of oil from carp (*Cyprinus carpio*) viscera. *Food Chemistry, 119*(3), 945-950.

Crivelli, A.J. (1981). The biology of the common carp, *Cyprinus carpio* L. in the Camargue, southern France. *Journal of Fish Biology, 18*, 271-290.

Crivelli, A.J. (1983). The destruction of aquatic vegetation by carp. *Hydrobiologia, 106*, 37-41.

Crooks, J.A. (2002). Characterizing ecosystem-level consequences of biological invasions: the role of ecosystem engineers. *Oikos, 97*, 153–166.

Crowder, L.B. & Cooper, W.E. (1982). Habitat structural complexity and the interaction between bluegills and their prey. *Ecology, 63*, 1802-1813.

Dembélé, K., Haubruge, E. & Gaspar, C. (2000). Concentration effects of selected insecticides on brain acetylcholinesterase in the common carp (*Cyprinus carpio* L.). *Ecotoxicology and Environmental Safety, 45*(1), 49-54.

Di Lullo, G.A., Sweeney, S.M., Körkkö, J., Ala-Kokko, L. & San Antonio, J.D. (2002). Mapping the ligand-binding sites and disease-associated mutations on the most abundant protein in the human, type I vollagen. *The Journal of Biological Chemistry, 277*(6), 4223-4231.

Doe, P.E. (Ed.). (1998). *Fish Drying and Smoking: Production and Quality*: CRC Press; Boca Raton.

Domínguez, P.L. (1988). Utilización de desperdicios alimentarios y de subproductos industriales agropecuarios y de la pesca en la alimentación del cerdo *En Alimentación porcina no convencional* (pp. 7-69). La Habana.

Driver, P.D., Closs, G.P. & Koen, T. (2005). The effects of size and density of carp (*Cyprinus carpio* L.) on water quality in an experimental pond. *Archiv für Hydrobiologie, 163*(1), 117-131.

Duan, R., Zhang, J., Du, X., Yao, X. & Konno, K. (2009). Properties of collagen from skin, scale and bone of carp (*Cyprinus carpio*). *Food Chemistry, 112*(3), 702-706.

Duman, M., Patir, B., Duman, E. & Ilhak, O. (2007). The effects of salt and storage temperature on microbiological changes in hot-smoked mirror carp *(Cyprinus carpio L.) Pakistan Journal of Biologial Science,.10*(17):3002-30055.

Enke, D.B.S., Lopes, P.S., Kich, H.A., Britto, A.P., Soquetta, M. & Pouey, J.L.O. F. (2009). Use of fish silage meal in diets for juvenile jundia fish. *Ciencia Rural, 39*(3), 871-877.

EW. (1999-2010). Lake Waikare. *Environment Waikato Regional Council*, 2010, from http://www.ew.govt.nz/environmental-information/Rivers-lakes-and-

wetlands/Learn-about-our-lakes/Shallow-lakes-of-the-Waikato-region/Riverine-lakes/Lake-Waikare/

Falfushynska, H.I. & Stolyar, O.B. (2009). Responses of biochemical markers in carp *Cyprinus carpio* from two field sites in Western Ukraine. *Ecotoxicology and Environmental Safety, 72*(3), 729-736.

FIN. (n.d.). Fishmeal for pigs- a feed with a very healthy future. Retrieved from http://www. iffo.net/intranet/content/archivos/85.pdf

FNZAS. (n.d.). National Aquatic Pest Taskforce. *Federation of New Zealand Aquatic Societies*, 2010, from http://www.fnzas.org.nz/index.php?PG= PestTaskForce

Folador, J.F., Karr-Lilienthal, L.K., Parsons, C.M., Bauer, L.L., Utterback, P.L., Schasteen, C.S., Bechtel, P.J. & Fahey, G.C. (2006). Fish meals, fish components, and fish protein hydrolysates as potential ingredients in pet foods. *Journal of Animal Science, 84*(10), 2752-2765.

Gálvez, A., Morales, L.J. & Bourges, R.H. (1985). Development of an enzymatic fish hydrolysate and its use in instant soup bases. *Archivos Latinoamericanos de Nutrición, 35*(4), 686-695.

García, D.M., Bauer, H., Dietz, T., Schubert, T., Markl, J. & Schaffeld, M. (2005). Identification of keratins and analysis of their expression in carp and goldfish: comparison with the zebrafish and trout keratin catalog. *Cell and Tissue Research, 322*(2), 245-256.

Gardeningzone. (2010). Hydrolyzed fish liquid pint. *Fish Hydrolysate 2-4-1*, 2010, from http://gardeningzone.com/product_info.php?products_id=197

Geirsdottir, M. (2009). Isolation, purification and investigation of peptides from fish proteins with blood pressure decreasing properties. Retrieved from http://www.matis.is/ media/ matis/utgafa/36-09-1787_Peptid.pdf

Gildberg, A. (1993). Review: Enzymic processing of marine raw materials. *Process Biochemistry, 28*, 1-15.

GMAP. (2005). The Superiority and Advantages of Gelatin, 2010, from http://www.gmap-gelatin.com/gelatin_adv.html

Golombieski, J.I., Marchesan, E., Camargo, E.R., Salbego, J., Schmitt Baumart, J.; Loro. V.L., Machado, S.L.O, Zanella. R., Baldisserotto, B. (2008). Acetylcholineseterase enzyme activity in carp brain and muscle after acute exposure to Diafuran. *Scientia Agricola, 65*(4), 340-345.

Gómez-Guillén, M.C., Giménez, B. & Montero, P. (2005). Extraction of gelatin from fish skins by high pressure treatment. *Food Hydrocolloids, 19*(5), 923-928.

Great Pacific Bioproducts Ltd. (2010). Why Use Fish Fertilizer, 2010, from http://www.greatpacificbioproducts.com/section.asp?pageid=12756

Green, S., Wiseman, J. & Cole, D.J.A. (1983). Fish silage in pig diets. *Pig news and information, 4*(3), 269-273.

Green, S., Wiseman, J. & Cole, D.J.A. (1988). Examination of stability, and its effect on the nutritive value, of fish silage in diets for growing pigs. *Animal Feed Science and Technology, 21*(1), 43-56.

Growth and Innovation Advisory Board. (2004). Research on Growth and Innovation: Research Summary. Retrieved from http://www.giab.govt.nz/ work-programme/growth/research-summary/ index.html

Hassan, R. (1994). Preparation and utilisation of fish waste silage with molasses as feed supplement for animals. Final Project Report to FAO, (Institut Agronomique et Vétérinaire, Morocco.

Hassan, T.E. & Heath, J.L. (1987). Chemical and nutritive characteristics of fish silage produced by biological fermentation. *Biological Wastes, 20*(3), 187-201.

Hicks, B.J., Ling, N., Osborne, M.W., Bell, D.G. & Ring, C.A. (2005). *Boat electrofishing Survey of the Lower Waikato River and its Tributaries.* Unpublished CBER Contract Report 39, University of Waikato, Hamilton.

Hinojosa-Garro, D. & Zambrano, L. (2004). Interactions of common carp (*Cyprinus carpio*) with benthic crayfish decapods in shallow ponds. *Hydrobiologia, 515*, 115–122.

Hume, D.J. & Pribble, H.J. (1980). The biology and behaviour of carp (*C. carpio* L.*)*: A brief review. *Australian Carp program Report No. 5*: Fisheries and Wildlife Division, Ministry of Conservation, Victoria.

Husain, R.A.K. & Offer, N.W. (1986). Effect of formaldehyde treatment on the degradation of acid-preserved fish silage protein in vitro. *Animal Feed Science and Technology, 16*(4), 297-304.

Ian, P. (n.d.). Health Benefits from feeding Fish Oil and Fish Meal, The Role of Long Chain Omega-3 Polyunsaturated Fatty Acids in Animal Feeding *IFOMA: Technical Bulletin No 28*.

Invasive Species Specialist Group (ISSG). (2000). IUCN Guideline for the Prevention of Biodiversity Loss Caused by Alien Invasive Species. *IUCN*. Gland, Switzerland.

Jónsson, Á., Finnbogadóttir, G.A., Porkelsson, G., Magnússon, H., Reykdal, Ó. & Arason, S. (2007). Dried fish as health food. Retrieved from http://www.matis.is/media/matis/utgafa/ Skyrsla_32-07.pdf

Jordan, R. (2006). Koi History - Myths and Mysteries, 2010, from http://www.mpks. org/articles/RayJordan/KoiHistory3.shtml

K&C Fisheries. (n.d.). Innovatively Transforming a 'Pest' to a 'Resource' for the Betterment of the Environment and the Community. Retrieved from http://www.kcfisheries.com.au/ companyoverview.htm

Kasankala, L.M., Xue, Y., Weilong, Y., Hong, S.D. & He, Q. (2007). Optimization of gelatine extraction from grass carp (*Catenopharyngodon idella*) fish skin by response surface methodology. *Bioresource technology, 98*(17), 3338-3343.

Kassuwi, S. (2009). *Biogas production from fish wastes in East African region*. Department of Biotechnology, Lund University. Sweden.

Kimura, S., Miyauchi, Y. & Uchida, N. (1991). Scale and bone type I collagens of carp (*Cyprinus carpio*). *Comparative Biochemistry and Physiology - Part B, 99*(2), 473-476.

Kimura, Y. (2002). Carp oil or oleic acid, but not linoleic acid or linolenic acid, inhibits tumor growth and metastasis in Lewis lung carcinoma-bearing mice. *Journal of Nutrition, 132*(7), 2069-2075.

Kjos, N.P., Herstad, O., Overland, M. & Skrede, A. (2000). Effects of dietary fish silage and fish fat on growth performance and meat quality of broiler chicks. *Canadian Journal of Animal Science*, 625-632.

Kompiang, I.P. (1981). Fish silage: its prospect and future in Indonesia. *Indonesia Agricultura Research and Development Journal, 3*, 9-12.

Kristinsson, H.G. & Rasco, B.A. (2000). Fish Protein Hydrolysates: Production, Biochemical and Functional Properties. *Food Science and Nutrition, 40*(1), 43-81.

Lamarra, V.A. (1975). Digestive activities of carp as a major contributor to the nutrient loading of lakes. *Verhandlungen Internationale Vereinigung für Theoretische und Angewandte Limnologie, 19*, 2461–2468.

Lammens, E.H.R.R. & Hoogenboezem, W. (1991). Diets and Feeding Behaviour. In: Winfield, I.J. & Nelson, J.S. (eds). *Cyprinid Fishes: Systematics, Biology and Exploitation*. Fish and Fisheries Series 3. Chapman and Hall, London, UK., 353–376.

Larsson, S.C., Bergkvist, L. & Wolk, A. (2006). Processed meat consumption, dietary nitrosamines and stomach cancer risk in a cohort of Swedish women. *International Journal of Cancer, 119*(4), 915-919.

Lin, P.Z., Zhang, J.S., Ding, Z.W. & Cai, H.Y. (1986). Carcinogenic and promoting effects of fish juice, preserved rice and salted dry fish on the

forestomach epithelium of mice and esophageal epithelium of rats. *Chinese Journal of Oncology, 8*(5), 332-335.

Living Countryside. (1999). Silage making, 2010, from http://www.ukagriculture.com/crops/silage_making.cfm

Lougheed, V., Crosbie, B. & Chow-Fraser, P. (1998). Predictions on the effect of common carp (Cyprinus carpio) exclusion on water quality, zooplankton, and submergent macrophytes in a Great Lakes wetland. *Canadian Journal of Fisheries and Aquatic Sciences, 55*, 1189-1197.

Maas-van Berkel, B., van den Boogaard, B. & Heijnen, C. (2004). *Agrodok 12: Preservation of Fish and Meat*: Agromisa Foundation, Wageningen.

Machin, D.H., Young, R.H. & Crean, K. (1982). The use of formic acid prepared fish silage made from shrimp by-catch in the diets of fattening pigs. *Tropical Animal Production, 7*, 120-126.

Malde, M.K., Graff, I.E., Siljander-Rasi, H., Venäläinen, E., Julshamn, K., Pedersen, J.I. & Valaja, J. (2010). Fish bones - a highly available calcium source for growing pigs. *Journal of Animal Physiology and Animal Nutrition, 94*(4). Availabe on line May 2010.

Manning, K. (2005). Why Fish Collagen Peptide Is Safe To Use. 2010, from http://www.articlesbase.com/health-articles/why-fish-collagen-peptide-is-safe-to-use-311287.html

McCrimmon, H.R. (1968). Carp in Canada. *Bulletin of the Fisheries Research Board of Canada, 165*, 1-93.

Meijer, M.L., De Boois, I., Scheffer, M., Portielje, R. & Hosper, H. (1999). Biomanipulation in shallow lakes in The Netherlands: an evaluation of 18 case studies. *Hydrobiologia, 408/409*, 13-30.

Miller, S.A. & Crowl, T.A. (2006). Effects of common carp (*Cyprinus carpio*) on macrophytes and invertebrate communities in a shallow lake. *Freshwater Biology, 51*, 85-94.

MINAG. (1975). *Instruction for the manufacture and use of the ensilage of fish in the cattle companies*. Ministry of the Agriculture of Cuba.

Moore, J.W., Schindler, D.E. & Scheuerell, M.D. (2004). Disturbance of freshwater habitats by anadromous salmon in Alaska. *Oecologia, 139*, 298–308.

Mostafaie, A., Bidmeshkipour, A., Shirvani, Z., Mansouri, K. & Chalabi, M. (2007). Kiwifruit actinidin: A proper new collagenase for isolation of cells from different tissues. *Applied Biochemistry and Biotechnology, 144*(2), 123-131.

Mshandete, A., Kivaisi, A., Rubindamayugi, M. & Mattiasson, B. (2004). Anaerobic batch co-digestion of sisal pulp and fish wastes. *Bioresource Technology, 95*(1), 19-24.

Murray, J. & Burt, J.R. (1979). *The Composition of Fish*. Ministry of Technology Torry Advisory Note 38, 11.

Murray, M. (2009). Amazing Fish Peptides Lower Blood Pressure. 2010, from http://www.doctormurray.com/index.php?option=com_content&view=article&id=139

Nagai, T. & Suzuki, N. (2000). Isolation of collagen from fish waste material - skin, bone and fins. *Food Chemistry, 68*(3), 277-281.

Nagai, T., Izumi, M. & Ishii, M. (2004). Fish scale collagen: Preparation and partial characterization. *International Journal of Food Science and Technology, 39*, 239–244.

NCC. (2009). Koi Carp. *Nelson City Council*, 2010, from http://www.nelsoncitycouncil.co.nz/koi-carp

NIF. (n,d). *Fish – Oil: Salmon oil*. Norwegian Institute of Fisheries, Aquaculture Research. Retrieved from http://www.rjtc.de/downloads/fish%20oil%20analysis %20results.pdf

Northland Regional Council. (2002). State of the environment 2002: Highlights from the Northland State of the Environment Report 2002. Whangarei, New Zealand: Northland Regional Council.

Nutrition Data. (2009a). Nutrition Facts and Analysis for Fish, cod, Atlantic, dried and salted. 2010, from http://nutritiondata.self.com/facts/finfish-and-shellfish-products/4044/2

Nutrition Data. (2009b). Nutrition Facts and Analysis for Fish, roe, mixed species, raw, 2010, from http://nutritiondata.self.com/facts/finfish-and-shellfish-products/4098/2

NZFWL. (1999). *Threats to native freshwater fish*. from New Zealand Native Freshwater Life website http://www.nzfreshwater.org/threats.html

Olitzky, I., Perri, A.M., Shiffman, M.A. & Werrin, M. (1956). Smoked fish as a vehicle of salmonellosis. *Public Health Reports*. *71*, 773-779.

Osborne, M.W. (2006). *Ecology of koi carp (Cyprinus carpio) in the Waikato River.* MSc thesis, University of Waikato, Hamilton, New Zealand.

Parkos, J.J., Santucci ., V.J. & Wahl, D.H. (2003). Effects of adult common carp (*Cyprinus carpio)* on multiple trophic levels in shallow mesocosms. *Canadian Journal of Fisheries and Aquatic Sciences*, *60*(2), 182-192.

Penedo, J.A., Cisneros, M.V. & Rodríguez, J.A. (1986). Ensilaje de pescado y ensilaje de pescado más miel final: características químicas y variaciones del pH. *Cuban Association for Animal Production, 5*(1), 22-24.

Pérez, R. (1988). El uso de las mieles de caña de azúcar en monogástricos. *Sugarcane as feed* (pp. 215-234): FAO Animal Production and Health Paper No. 72. Rome, FAO.

Pérez, R. (1995). Better feed for animals: more food for people. *World Animal Review*(82).

Persson, L. (1983). Food consumption and competition between age classes in a perch *Perca fluviatilis* population in a shallow eutrophic lake. *Oikos*, *40*, 197–207.

Persson, L. (1985). Asymmetrical competition: are larger animals competitively superior? *The American Naturalist, 126*, 261–266.

Picot, L., Bordenave, S., Didelot, S., Fruitier-Arnaudin, I., Sannier, F., Thorkelsson, G., Bergéc, P., Guérardd, F., Chabeaudd, A, & Piota, J.M. (2006). Anti-proliferative activity of fish protein hydrolysates on human breast cancer cell lines. *Process Biochemistry, 41*(5), 1217-1222.

Polis, G.A. (1988): Exploitation competition and the evolution of interference, cannibalism, and intraguild predation in age/size structured populations. In: Ebenman, B. & Persson, L. (eds). *Size-Structured Populations. Ecology and Evolution*. Springer-Verlag, Berlin, Germany, pp. 185–202.

Pommet, M., Redl, A., Morel, M.-H., Domenek, S. & Guilbert, S. (2003). Thermoplastic processing of protein-based bioplastics: chemical engineering aspects of mixing, extrusion and hot molding. *Macromolecular Symposia, 197*, 207-217.

Prescott. L., Harley, J., & Klein, D. (1999). *Micorbial Growth* In: Microbiology 4th edn. chapt 6 Retrieved from http://www.biologie.uni-hamburg.de/b-online/ library/micro229/terry/229sp00/ lectures/growth.html

Préstamo, G. (1993). Actinidin in kiwifruit cultivars. *Zeitschrift für Lebensmittel-untersuchung und -Forschung A, 200*(1), 64-66.

Pullan, S.G. (1984). Japanese koi (*Cyprinus carpio)* in the Waikato River system. *New Zealand Ministry of Agriculture and Fisheries, Report No. 2*. Auckland.

Purves, D., Augustine, G. J., Fitzpatrick, D., Hall, W.C., LaMantia, A.S., McNamara, J.O., Willams, S.M.. (2008). *Neuroscience* 4th edn.: Sinauer, Sunderland.

Rai, A.K., Swapna, H.C., Bhaskar, N., Halami, P.M. & Sachindra, N.M. (2010). Effect of fermentation ensilaging on recovery of oil from fresh water fish viscera. *Enzyme and Microbial Technology, 46*(1), 9-13.

Rashed, M. N. (n.d.). *Biomarkers as indicator for water pollution with heavy metals in rivers, seas and oceans*. 2010, from http://www.wrrc.dpri.kyoto-u.ac.jp/~aphw/APHW2004/proceedings/OHS/56-OHS-A344/56-OHS-A344.pdf

Rattagool, P., Surachatmrongratane, S. & Wongchinde, N. (1980). Fish silage in Thailand. Further trials on broiler chicken. *Proc. IPFC Workshop Fish Silage* (pp. 59-63). Rome.

Reece, P. (2006). Recovery of high quality oil from mackerel and sprat by the silage process. *Journal of the Science of Food and Agriculture, 32*(6), 531-538.

Reyes, G., Martinez, R., Rodriguez, L. M., Bello, R. A. & Cruz-Pascual, M. (1991). Effect of the addition of tropical fruit wastes on the rate of production of microbial silage from fish. *Alimentaria, 28*, 219, 299-108.

Roberts, J., Chick, A., Oswald, L. & Thompson, P. (1995). Effect of carp, *Cyprinus carpio*, an exotic benthivorous fish, on aquatic plants and water quality in experimental ponds. *Marine and Freshwater Research, 46*, 1171-1180.

Robinson, P., Beckley, M. A., Neale, M. & Ormsby, W. (2007). Process of preparing fertilizer by acid hydrolysis of fish. CA 2,546,156.

Rodríguez, T., Montilla, J.J. & Bello, R. A. (1990). Fish silage prepared from fish species of shrimp by-catch. II. Biological test in broilers. *Archivos Latinoamericanos de Nutrición, 40*(4), 548-559.

Salgado-Maldonado, G. & Pineda-López, R.F. (2004). The Asian fish tapeworm *Bothriocephalus acheilognathi*: A potential threat to native freshwater fish species in Mexico. *Biological Invasions, 5*(3), 261-268.

Santoul, F. & Mastrorillo, S. (2003). Interaction between fish and waterbird communities: A case study of two gravel pits in south-west France. *Vie et Milieu-life and Environment, 53*, 131-133.

Schachter, H., Reisman, J., Tran, K., Dales, B., Kourad, K., Barnes, D., Sampson, M., Morrison. A., Gaboury, I. & Blackman, J. (2004). Health Effects of Omega-3 Fatty Acids on Asthma. *Evidence Report/Technology Assessment No. 91*. Ottawa, Canada: University of Ottawa Evidence-based Practice Center.

Schaus, M.H. & Vanni, M.J. (2000). Effect of gizzard shad on phytoplankton and nutrient dynamics: role of sediment feeding and fish size. *Ecology, 81*, 1701–1719.

Scheffer, M., Carpenter, S., Foley, J.A., Folke, C. & Walker, B. (2001). Catastrophic shifts in ecosystems. *Nature, 413*, 591-596.

Schroeder, J.W. (2004). Quality Forage: Silage Fermentation and Preservation *AS-1254*. Fargo, North Dakota: North Dakota State University.

Sehgal, H.S., Shahi, M., Sehgal, G.K. & Thind, S.S. (2008). Some quality aspects of fish patties prepared from an Indian major carp, *Labeo rohita* (Ham.). *International Journal of Food Sciences and Nutrition, 59*(3), 192-201.

Sibbing, F., Osse, J. & Terlouw, A. (1986). Food handling in the carp (*Cyprinus carpio)*: its movement patterns, mechanisms and limitations. *Journal of Zoology, 210*(A), 161-203.

Simopoulos, A.P. (2002). The importance of the ratio of omega-6/omega-3 essential fatty acids. *Biomedicine and Pharmacotherapy, 56*(8), 365-379.

Sinclair, A.J., Oon, K.S., Lim, L., Li, D. & Mann, N.J. (1998). The omega-3 fatty acid content of canned, smoked and fresh fish in Australia. *Australian Journal of Nutrition and Dietetics, 55*(3), 116-120.

Stecker, M.A. (2007). New Zealand Home Page, 2010, from http://mstecker.com/home1.htm

Stuart, I. & Jones, M. (2002). Ecology and Management of Common Carp in the Barmah-Millewa Forest. *Final report of The Point Source Management of Carp Project to Agriculture Fisheries and Forestry Australia*: Arthur Rylah Institute, Victoria.

Suarez, A.M., Batarlo, J.A.C., Afable, R.M.F., Cubos, K.R.S., De Leon, A.J.R., Eyo, M.L.A.P. & Manalad, A.L.M. (2009). Bioplastics from Fish Scales. Retrieved from http://www.scribd.com/doc/30495324/Bio-Plastics-From-Fish-Scales

Sullivan, A.O., Shaw, N.B., Murphy, S.C., van de Vis, J.W., van Pelt-Heerschap, H. & Kerry, J.P. (2006). Extraction of collagen from fish skins and its use in the manufacture of biopolymer films. *Journal of Aquatic Food Product Technology, 15*(3), 21-32.

Swan, J.E. (2000). Animal by-product processing. In: F.J. Francis (Ed.), *Encyclopedia of Food Science and Technology* (2nd edn., pp. 35-43). New York: John Wiley.

Synnes, O.M. & Opstad, S. (1995). Effect of fish silage on yield and chemical composition of Italian ryegrass (*Lolium multiflorum* Lam.). *Acta Agriculturae Scandinavica, Section B - Plant Soil Science, 45*(1), 68-72.

Szabó, A., Nemcsok, J., Asztalos, B., Rakonczay, Z., Kása, P. & Hieu, L.H. (1992). The effect of pesticides on carp (*Cyprinus carpio* L).

Acetylcholinesterase and its biochemical characterization. *Ecotoxicology and Environmental Safety, 23*(1), 39-45.

Tatterson, I. N. & Windsor, M. L. (2006). Fish Silage. *Journal of the Science of Food and Agriculture, 25*, 369-379.

Tempero, G.W., Ling, N., Hicks, B.J. & Osborne, M.W. (2006). Age composition, growth, and reproduction of koi carp (*Cyprinus carpio*) in the lower Waikato region, New Zealand. *New Zealand Journal of Marine and Freshwater Research, 40*, 571–583.

The University of Waikato. (2005). What is silage? *The New Zealand Biotechnology Learning Hub*. Retrieved from http://www.biotechlearn. org.nz/themes/future_ farming/what_is_silage

Thomas, M. & Engle, C.R. (1995). Consumer acceptance of canned bighead carp: a new freshwater fish product. *Arkansas Agricultural Experiment Station Report Series 328*.

Tibbetts, G.W., Seerly, R.W., McCampbell, H.C. & Vezey, S.A. (1981). An evaluation of an ensiled waste fish product in swine diets. *Journal of Animal Science, 52*, 93-100.

TDRI (Tropical Development and Research Institute). (1982). Fish silage. *Fish Handling, Preservation and Processing in the Tropics: Part 2*. London.

Tucker, C.S. (2000). Off-flavor problems in Aquaculture. *Reviews in Fisheries Science, 8*(1), 45-88.

UMMC. (2010). Maryland Parkinson's Disease and Movement Disorders Center. *University of Maryland Medical Center*, 2010, from http://www.umm.edu/ parkinsons/signs.htm

United Fisheries. (2010). *Liquid Fish Silage Reduces Greenhouse Gas Emissions*, from http://www.unitedfisheries.co.nz/content/liquid-fish-silage

University of Waikato. (2007). New Zealand's unique ecology. *Science Learning Hub*, 2010, from http://www.sciencelearn.org.nz/Contexts/Hidden-Taonga/ Looking-Closer/New-Zealand-s-unique-ecology

Ünlüsayn, M., Kaleli, S. & Gulyavuz, H. (2001). The determination of flesh productivity and protein components of some fish species after hot smoking. *Journal of the Science of Food and Agriculture, 81*(7), 661-664.

van der Oost, R., Lopes, S.C.C., Komen, H., Satumalay, K., van den Bos, R., Heida, H. & Vermeulen, N.P.E. (1998). Assessment of environmental quality and inland water pollution using biomarker responses in caged carp (*Cyprinus carpio*): Use of a bioactivation:detoxication ratio as a Biotransformation Index (BTI). *Marine Environmental Research, 46*(1-5), 315-319.

Venugopal, V. (2009). *Marine Products for Healthcare: Functional and Bioactive Nutraceutical Compounds from the Ocean*. Boca Raton, Florida: Taylor & Francis.

Vidotti, R.M., Viegas, E.M.M. & Carneiro, D.J. (2003). Amino acid composition of processed fish silage using different raw materials. *Animal Feed Science and Technology, 105*, 199–204.

Wang, L., Yang, B., Wang, R. & Du, X. (2008). Extraction of pepsin-soluble collagen from grass carp (*Ctenopharyngodon idella*) skin using an artificial neural network. *Food Chemistry, 111*(3), 683-686.

Watkins, B.A., Chwan-L, S., Allen, K.G.C. & Seiffert, M.F. (1996). Dietary (n-3) and (n-6) polyunsaturates and acetylsalicylic acid alter ex-vivo PGE2 synthesis, tissue IGF-1 levels and bone morphology in chicks. *Journal of Bone and Mineral Research, 11*, 1321-1332.

Watts, C. & Peters, M. (2010). *Pests.* In: Wetland Restoration: A Handbook for NZ Freshwater Systems. M. Peters & B. Clarkson (Eds.)

Welcomme, R.L. (1992). A history of international introductions of inland aquatic species. *ICES Marine Science Symposia*. Copenhagen, Denmark.

Whittemore, C.J. & Taylor, A.G. (1976). Nutritive value to the growing pig of de-oiled liquefied offal preserved with formic acid (fish silage). *Journal of the Science of Food and Agriculture, 27*, 239-243.

Wikipedia. (2010b). cycloid scale, 2010, from http://en.wikipedia.org/wiki/Scale _% 28zoology%29

Wikipedia. (2010a). Taramosalata, 2010, from http://en.wikipedia.org/wiki/ Taramosalata

Wiseman, J., Green, S. & Cole, D.J.A. (1982). *Nutritive value of oily-fish silage when used to replace ingredients of plant origin in growing pig diets.* Paper presented at the Proceedings of the British Society of Animal Production.

Woo, J.W., Yu, S.J., Cho, S.M., Lee, Y.B. & Kim, S.B. (2008). Extraction optimization and properties of collagen from yellowfin tuna (*Thunnus albacares*) dorsal skin. *Food Hydrocolloids, 22*(5), 879-887.

Yang, J.G., Li, G.L., Jin, Q.H., Chen, D.H. & He, S.W. (1994). Determination of the contents of EPA and DHA and lipid constituents in main fresh water fishes in China. *Journal of Tongji Medical University, 14*(2), 77-80.

Yankah, V.V., Ohshima, T.,H. Ushio, H., Fujii, T. & Koizumi, C. (1996). Study of the differences between two salt qualities on microbiology, lipid, and water-extractable components of Momoni, a Ghanaian fermented fish product. *Journal of the Science of Food and Agriculture, 71*, 33-40.

Zambrano, L. & Hinojosa-Garro, D. (1999). Direct and indirect effects of carp (*Cyprinus carpio* L.) on macrophyte and benthic communities in experimental shallow ponds in central Mexico. *Hydrobiologia, 408/409*, 131-138.

Zambrano, L., Scheffer, M. & Martı´nez-Ramos, M. (2001). Catastrophic response of lakes to benthivorous fish introduction. *OIKOS, 94*, 344-350.